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Virus Inactivation in Wastewater Effluents by Chlorine, Ozone, and Ultraviolet Light

Radian Corp.
Austin, TX

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VIRUS INACTIVATION IN WASTEWATER EFFLUENTS BY CHLORINE, OZONE, AND ULTRAVIOLET LIGHT

by

R. A. Fluegge
Radian Corporation
Austin, Texas 78766

T. G. Metcalf
Dept. of Microbiology, Univ. of New Hampshire
Durham, New Hampshire 03824

C. Wallis
Dept. of Microbiology, Baylor University
Houston, Texas 77025

Grant No. R804991

Project Officer
Albert D. Venosa
Wastewater Research Division
Municipal Environmental Research Laboratory
Cincinnati, Ohio 45268

MUNICIPAL ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

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16. ABSTRACT

In this investigation four wastewater treatment plants were studied and compared in their ability to inactivate naturally occurring enteroviruses. Of the four plants tested, two used chlorine as the disinfectant, one used ozone, and one used ultraviolet light.

Results of field testing are summarized as follows. Both virus titers and isolation rates (percentrage of samples positive for virus) were low in the undisinfected effluents. As a result there was no consistent correlation between virus numbers and any of the traditionally measured bacteriological indicators. Also, no seasonal variation in virus titers was detected in any of the effluents. However, a diurnal variation was observed in one of the plants during two separate samplings.

Virus titers and isolation rates in the disinfected effluents were significantly lower than in the undisinfected controls. However, the numbers were too low to detect differences between plants or disinfectants.

At least 10 different virus types were isolated in the tested effluents. The predominant virus type was poliovirus 1.

Seeding experiments with attenuated strains of poliovirus were performed to evaluate the recovery efficiency in each effluent. Efficiencies were low and markedly variable.

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimonies to the deterioration of our natural environment. The complexity of that environment and the interplay of its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution; and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems to prevent, treat, and manage wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, to preserve and treat public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research and provides a most vital communications link between the researcher and the user community.

This report is a summary of findings from a field and laboratory program to determine the virucidal effects of chlorine, ozone, and ultraviolet light under actual field conditions. The effluents from four publicly owned wastewater treatment plants were sampled, studied, and the results presented herein.

Francis T. Mayo
Director
Municipal Environmental
Research Laboratory

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ABSTRACT

This research program was designed to compare the abilities of chlorine, ozone, and ultraviolet light to inactivate naturally occurring enteroviruses in wastewater effluent. Of the four treatment plants tested, two used chlorine as the disinfectant, two (one full scale; one pilot) used ozone, and one used ultraviolet light.

Results for the viral content in wastewater effluents indicate no consistent correlation between virus concentrations and any of the "traditionally" measured indicators of pollution. In fact, the picture is very confusing with reverse roles found for several parameters, in particular total and fecal coliforms. Data for Muddy Creek Treatment Plant are a strong indication that no consistent correlation exists between virus concentrations and coliform concentrations. The experimental studies in Colorado show that absorbed dosages of ozone above 4 to 5 mg/l are required to ensure virus die-off.

No real seasonal variation of viruses was detected. However, a diurnal variation was found at one plant during two separate studies; one in December and one in July. Maximum virus concentrations occurred between 2:00 AM and 4:00 AM for each study.

At least 10 different virus types were found in the wastewater effluents.

Procedures and results of seeded virus studies designed to test the efficiency of virus recovery from the various test effluents are included. These runs were performed using known, pure strains and quantities of non-virulent viruses and were intended to approximate real world conditions. The results indicate that variability in viral seed recovery data, while common in a controlled laboratory environment, is more marked in the field situations.

This report is submitted in fulfillment of Grant No. R804991 by The Carborundum Company under the sponsorship of the the U. S. Environmental Protection Agency. This report covers the period September 27, 1976 to December 31, 1979. The experimental work was completed as of January 31, 1979.

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SECTION I

INTRODUCTION

Municipal wastewaters contain a large variety of potentially infectious microorganisms. These pathogenic organisms dispersing in the public waterways are capable of initiating disease epidemics. Disinfection of the effluents from wastewater treatment plants to inactivate disease-producing organisms is critical for the maintenance of the public health where there is the possibility of human contact.

The primary disinfectant utilized by Publicly Owned Treatment Works (P.O.T.W.) in the United States is chlorine. Environmentalists have recently been concerned about the adverse effects on water ecology produced by chlorine residuals in wastewater plant effluents. Scientific disapproval has increased with the knowledge that chlorine reacts with other substances to produce compounds shown to be carcinogenic to laboratory animals.

Viruses are considered by many to be the dominant hazard from wastewater disposal. Viruses have been demonstrated to be more resistant to disinfection processes than fecal coliform bacteria (Bates et al., 1977; Hoehn, 1976; White, 1972), the "traditional" indicator organisms. Hence, monitoring for the presence or absence of viruses in wastewater effluents is desirable and may well be a necessary addition to monitoring for fecal coliform bacteria or other indicator organisms.

Several laboratory studies (Vaughn and Metcalf, 1974; Scarpino, 1978) have been conducted to determine whether some indicator organisms or parameters other than fecal coliforms may be substituted for natural virus determinations. The results of these studies have been inconclusive.

Studies which involve the introduction of naturally occurring viruses into normal wastewater effluent streams or other waters contacted by the public are not usually conducted. To perform these studies, significant concentrations of natural viruses would have to be placed in the test stream. The risk to public health by such experimentation is not justified.

The present study was designed to test the relative effectiveness of chlorine, ozone, and ultraviolet light in

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inactivating viruses from the effluents of wastewater treatment plants. In addition to comparing the three disinfection procedures, the data were used to indicate any possible correlations between the currently measured wastewater parameters or indicator organisms, and natural virus levels. The information gained can be used to supply data useful in the determination of the safest and most economically feasible disinfection techniques.

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SECTION 2
CONCLUSIONS

At least ten different virus types in varies concentrations were isolated from both treated and treated plus disinfected effluents from four P.O.T.W. facilities. The predominant virus type found in both the treated and treated plus disinfected effluents was poliovirus 1.

Only small differences were indicated in seasonal isolation rates for viruses. The similarities were graphically evident for the July and December sampling periods at the Muddy Creek Works where the two isolation rates were 75 per cent and 60 percent, respectively.

The virus recovery results from the Muddy Creek Works reveal that virus concentrations vary diurnally. In this instance, virus recoveries were significantly greater between 2:00 AM and 4:00 AM in both December and July. This increase remains unexplained at this time.

The test results indicate that the parameters which are classically used to measure the effectiveness of disinfection of P.O.T.W. effluents are inadequate indicators of the viral content in these same effluents. Either other parameters must be developed which would correlate accurately to the presence and concentration of viruses, or viruses (virus monitoring) alone should be used as an indicator of a potentially hazardous discharge into the environment.

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SECTION 3

RECOMMENDATIONS

It would be of interest and importance to determine if the polio 1 viruses recovered from the wastewater plant effluents were of a virulent (wild) strain or an attenuated (vaccine) strain. This information would be of special value if any of the disinfectants was shown to be selectively effective in eliminating the wild or virulent strain.

It is recommended that future, similar experiments use T Marker tests. The "$T_{40}{}^{o}C$" marker (T) test could be performed in vitro on the poliovirus 1 isolates to determine virulence. This test refers to the ability of virulent (T+) isolates to grow as well at 40°C as at the normal (36°C) incubation temperature, while the attenuated (T-) strain shows a marked reduction in titer at this elevated temperature. This test can be used to classify any particular poliovirus 1 isolate in relations to known virulent and non-virulent strains.

Additional testing is required to indicate whether significant differences in the seasonal isolation rates for viruses occur.

The significance of diurnal variations in the virus input levels to a wastewater treatment plant as revealed at the Muddy Creek Works is important to determine disinfection levels required. Comparative data from other water works are required to test this finding.

In general, although the present study provides a strong framework for an analysis of the effectiveness of the various disinfectants in inactivating viruses, questions in many areas remain unanswered or unconfirmed. The amount of data required to provide a statistically accurate profile on the relative disinfection abilities of chlorine, ozone, and ultraviolet radiations is attainable only if further sampling studies are undertaken as part of a coordinated research program. With the accumulation of enough data points, statistics become meaningful, and questions about the relative efficacies of treatment are more easily answered. To this end, an increase in the number of virus sampling programs is highly recommended in order to obtain the data necessary to determine statistically definitive differences among methods of disinfection.

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SECTION 4

WASTEWATER TREATMENT PLANT DESCRIPTIONS

GENERAL DESCRIPTION

Four treatment plants were studied during this program. A total of five separate sample collection periods were completed between November 1976 and May 1978. The sampling sites were located at points immediately before and after the disinfectants studied.

Muddy Creek Works

The Muddy Creek Works, Cincinnati, Ohio is a secondary treatment facility using a conventional activated sludge system followed by disinfection with chlorine. Muddy Creek undisinfected effluent was transported to the Robert A. Taft Laboratory pilot plant in December, 1976 and treated with ozone. Undisinfected effluent was tested at the main plant in July, 1977 after treatment with chlorine. A block diagram of this plant is shown in Figure 1.

Raw wastewater flowed through a flood control chamber prior to entering the pump building which housed a screening device, a wet well, and raw wastewater pumps. After passing through a screen where large objects were removed, the wastewater entered the wet well, where it was raised by centrifugal pumps to a rising well. From the rising well, the raw wastewater flowed by gravity through the remaining treatment process.

Wastewater flowed through detritus tanks which removed sand, cinders and coarse grit before it passed through the three comminutors, which reduced the size of coarse solids to sizes less than 23 mm. The wastewater then flowed into the pre-aeration tanks. These tanks were tri-functional units since they also aided in the removal of fine, gritty material. The wastewater odors were controlled by bubbling air, which was also used for mixing the chemicals for phosphorus removal.

After leaving the pre-aeration tanks, the wastewater continued into the primary settling tanks where 60 percent of the suspended solids settled to the bottom. Primary or raw sludge was mechanically scraped and pumped to sludge holding tanks. The settled wastewater passed through a secondary metering chamber into aeration tanks. It was then mixed with the activated sludge, which was returned from the

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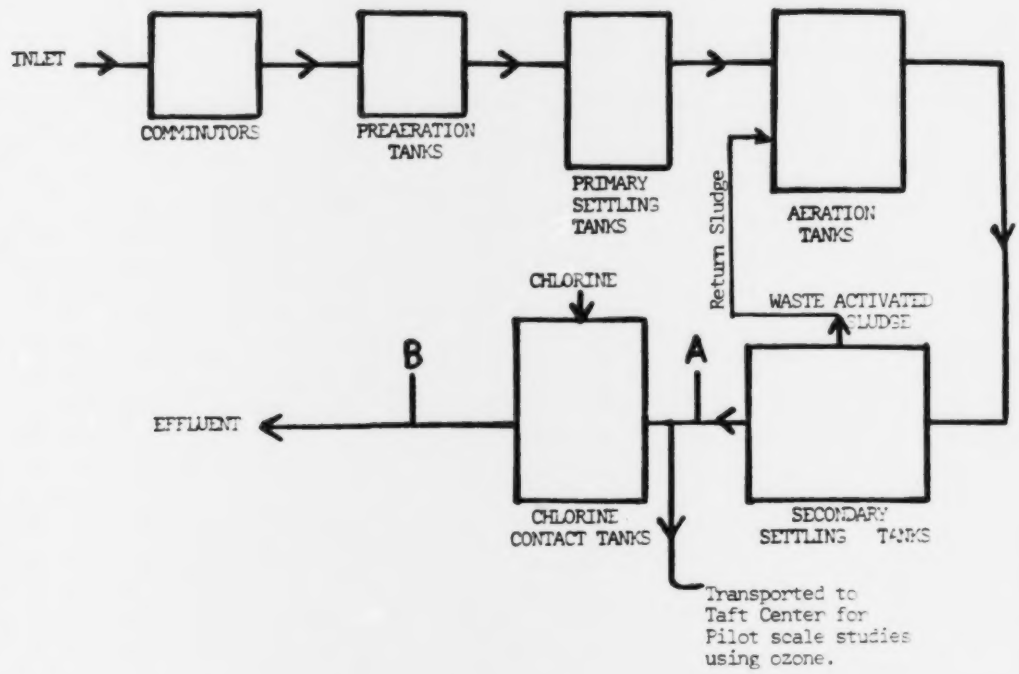

Design: Ave Flow (56,800m^3/d) 15 mgd
Sludge Dewatering: Vacuum Filtration
Disinfectant: Chlorine
Sampling Point A: 91.5m Upstream of Contact Tank
Sampling Point B: Effluent of Chlorine Contact Tanks

Figure 1. Muddy creek works and sampling sites.

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secondary settling tanks. Air was supplied by blowers located on one side near the bottom of each aeration tank, which caused a spiral flow mixing action as well as oxygenation.

After a detention time of 6 hours in the aeration tanks, the mixed liquor entered the secondary settling tanks where the activated sludge settled to the bottom and was returned to the aeration tanks. The treated wastewater from the secondary settling tanks flowed over weirs to the chlorine contact tank. The disinfected wastewater then flowed through the plant effluent line into Muddy Creek and on into the Ohio River. The chlorine dose added produced the following:

- Average total chlorine residual: 1.2 mg/l
- Contact time: 50 minutes

Both the raw sludge and scum from the primary settling tanks as well as that held in the sludge holding tanks underwent thermal sludge conditioning (heat and pressure), dewatering by vacuum filtration, incineration, and deposition as a slurry of ash and water into a diked lagoon.

Sample Point A: Secondary Effluent--

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The sampling point for the plant effluent prior to treatment by chlorine was located at a manhole approximately 6.1m (20 ft.) from the effluent of the secondary settling tanks and approximately 91.5m (300 ft.) upstream of the chlorine contact chamber.

Sample Point B: Chlorinated Secondary Effluent--

Sampling site for monitoring disinfection by chlorine was located at a manhole about 3.0m (10 ft.) from the effluent of the chlorine detention tanks.

Ozonated Secondary Effluent--

Ozone was incorporated as an alternative disinfectant for part of this study. Treated secondary effluent from sample point A was transported in a $20m^3$ (5400 gal.) epoxy-lined tank truck to EPA's Taft Research Center. This effluent was pumped into the pilot plant and through a packed column ozone contact tank at a rate of 75 l/min. (20 gal./min.). This provided an average contact time of 30 s. Sampling commenced immediately after passage of the effluent through this column. The average ozone dose was 8 mg/l and the residual was 0.13 mg/l.

A full description of this pilot facility is given by Venosa, et al., 1979.

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Upper Thompson Sanitation District Treatment Plant (UTSD)

The UTSD Treatment Plant, Estes Park, Colorado, consists of a conventional activated sludge plant followed by attached growth nitrification, tri-media filtration, and disinfection by ozone (air is used for ozone production). It was sampled during the spring of 1977. A block diagram of this plant is shown in Figure 2.

Raw wastewater entering the plant passed through a bar screen and comminutors where large solids were removed and/or reduced in size. It then entered an aeration and degrit tank where grit (sand, small solid particles, etc.) was removed by settling. The degritted effluent entered an equalization basin which was designed to maintain a constant flow before entry into the activated sludge tank. Mixing also took place in the equalization chamber to insure homogeneity.

Return activated sludge was mixed with equalization basin effluent and the resultant mixed liquor was aerated and, subsequently, drawn off into a clarifier. The biological solids formed during aeration were allowed to settle and the liquid supernatant pumped to the attached growth nitrification towers. Alum was sometimes added at the clarifier stage to aid in flocculation and the settling of solids.

A portion of the settled sludge was returned to the activated sludge tank. The waste activated sludge underwent further oxidation in an aerobic digester, then mechanical dewatering and thickening, and finally, movement to a sanitary landfill. Nitrification by biological growths was used to convert the ammonia present into nitrates. Nitrifying bacteria grew on stable surfaces within a media packed tower and the wastewater flow trickled over them.

After nitrification, the effluent underwent filtration to remove the remaining organic solids. Alum and sometimes a polyelectrolyte were added to the wastewater to flocculate the remaining suspended solids. Filtration was accomplished by passing the water through a tri-media filter bed. This filter bed consisted of several layers of progressively finer granular materials (anthracite, sand, garnet) which removed the flocculated solids by physical screening and interparticle action. The captured solids were removed from the filter bed by backwashing. This water was returned to the equalization chamber and recycled through the treatment system.

The final treatment step was ozone disinfection. Ozone was produced from air using a water-cooled, corona discharge generator. The contactor was a baffled, closed, rectangular

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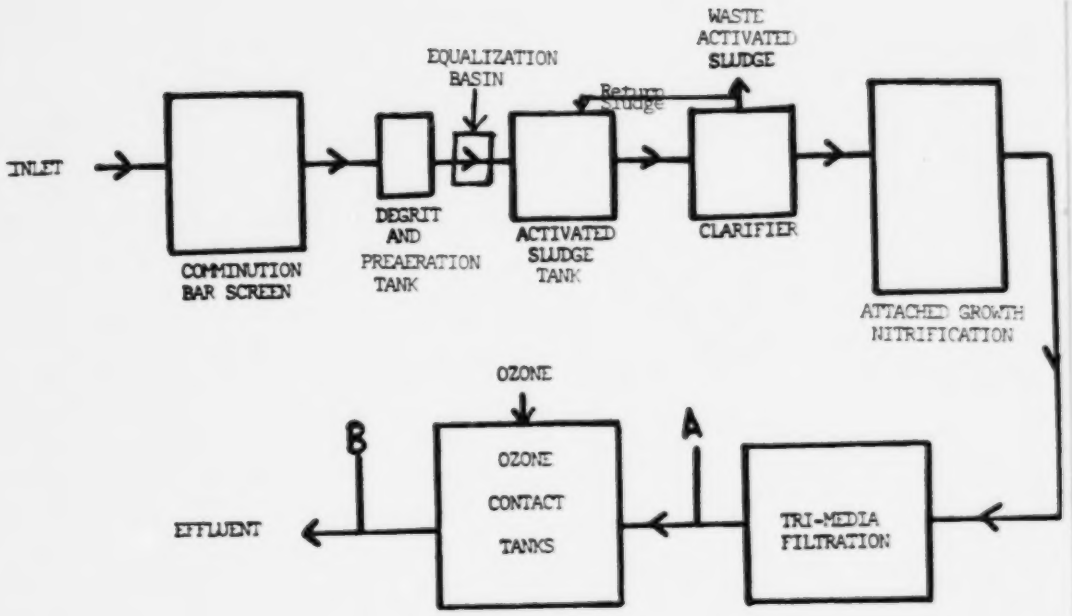

Design: Avg. Flow 5,680m^3/d (1.5 mgd)
Sludge Dewatering: Pressure Rollers
Disinfectant: Ozone
Sampling Point A: After the Tri-Media Filter
Sampling Point B: Effluent from Ozone Contact Tank

Figure 2. UTSD treatment plant and sampling sites.

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tank in which effluent flowed concurrent and countercurrent to the ozone. Ozone was injected through porous stone diffusers placed at the bottom of the tank. The contact time at average flow was 37 minutes.

The final effluent entered the Big Thompson River below Olympus Dam.

Sample Point A: Tertiary Treated Effluent Sampling Site--

The treated effluent was drawn from one of two tri-media filters. Point A was a water tap located between the tri-media filter and a flow regulating valve. This valve acted as a physical, water sealed barrier to gaseous ozone.

Sample Point B: Tertiary Treated and Ozonated Effluent Sampling Site--

Disinfected samples were drawn using a submersible, electric pump placed in the exit from the ozone contact basin. The basin was located beneath the plant administration offices and laboratories.

Marlborough Easterly Wastewater Plant

The Marlborough Easterly Wastewater Plant, Marlborough, Massachusetts, used a two-stage activated sludge system for nitrification followed by disinfection with chlorine. Effluents from this plant were collected and studied in October 1977. A block diagram of this plant is shown in Figure 3.

Raw wastewater entered the plant through a bar screen and comminutors where large solids were removed or reduced in size.

After flowing through the comminutors, it then passed through aerated grit chambers, where grit was removed by controlled settling. The degritted wastewater flowed into primary setttling tanks where the heavier organic and inorganic solids were separated from the wastewater. The raw, primary sludge was pumped to the operations building for processing and/or to sludge storage tanks. Bouyant materials were also removed at this point.

From the primary settling tanks, the wastewater flowed to the first stage aeration tanks. In the interconnecting pipes, activated sludge was added and mixed with the primary effluent before reaching the aeration tanks. The wastewater and activated sludge (mixed liquor) were kept mixed and aerated with oxygen by agitation with mechanical aerators. Following a detention period in the aeration tanks, the mixed liquor flowed to the first stage settling tanks where the activated sludge

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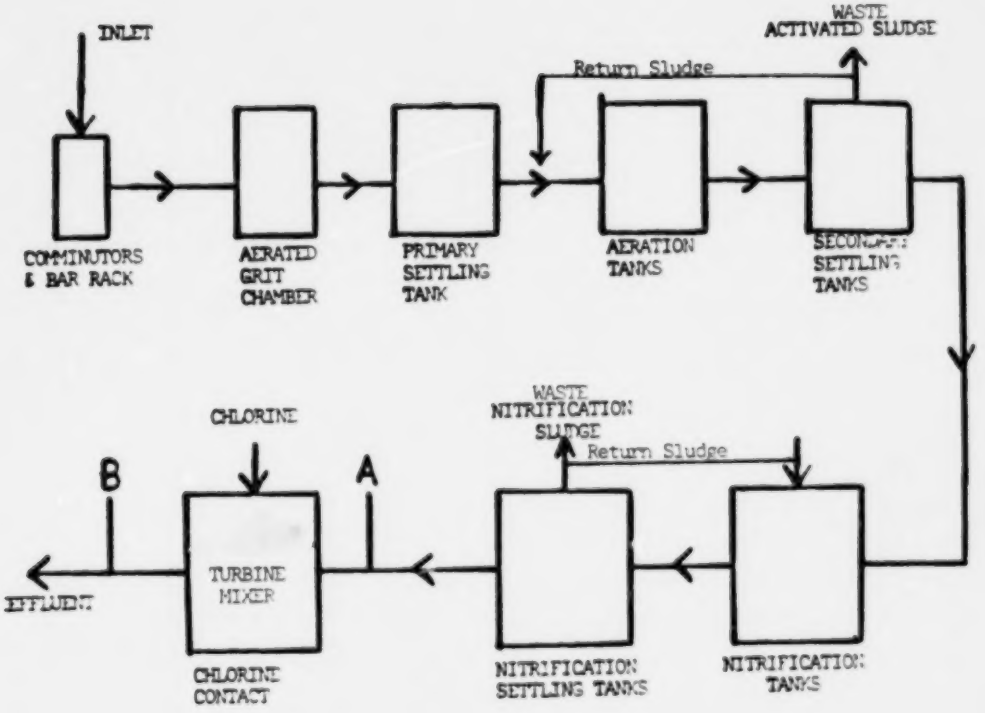

Design: Avg. Flow 5.5 mgd (20,800m^3/d)
Sludge Dewatering: Vacuum Filtration
Disinfectant: Chlorine
Sampling Point A: 22.9m Upstream of Contact Tank
Sampling Point B: Effluent of Contact Tank

Figure 3. Marlborough Easterly Wastewater Plant and sampling sites

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settled from the wastewater. Most of the activated sludge was returned to the aeration tanks. However, on an as needed basis, some sludge was washed to the primary settling tanks and discarded as primary sludge.

Phosphorus removal was required and was performed in the first stage of the activated sludge system. The phosphorus removal was accomplished by chemical precipitation with alum. The alum was introduced and mixed with liquor flow in the aeration tank effluent channel. The resultant insoluble phosphorus was removed in the secondary settling tanks along with the activated sludge and recycled back to the aeration tanks. After undergoing first stage activated sludge treatment, the first stage effluent can be directed either to the chlorine contact tanks for disinfection and discharge into Hop Brook or to the second stage nitrification activated sludge system. The latter situation was the case for these studies.

The nitrification stage is basically a biological process in which bacteria convert ammonia to nitrates. Mechanical aerators were used in the nitrification tanks to keep the first stage effluent and nitrification sludge well mixed and aerated with oxygen. The nitrification tank effluent flowed to the nitrification settling tanks where the nitrification sludge was separated from the main wastewater flow and recirculated to the nitrification tanks or wasted to the primary settling tanks.

The nitrified effluent then flowed to the chlorine contact tanks where chlorine was added and mixed with a turbine mixer for disinfection. The average total chlorine residual was 1.3 mg/l after a contact time of 50 minutes.

After leaving the chlorine contact tanks, the final effluent was reaerated by cascading down hydraulic jump steps leading to the plant outfall.

Sludge and scum from the primary settling tanks were pumped either to the storage tanks or to the operations building for processing. The sludge processing consisted of chemical conditioning dewatering on vacuum filters, and disposal in a sanitary landfill.

Sample Point A: Nitrified Effluent Sampling Site--

This source was located at a point 22.9m (75 ft.) upstream from the chlorination chamber and 7.6m (25 ft.) downstream of the nitrification settling tanks.

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Sample Point B: Chlorinated Nitrified Effluent Sampling Site--

This source was located at the effluent from the chlorine contact chambers and just upstream from the aeration steps.

Northwest Bergen County Treatment Plant

The Northwest Bergen County Treatment Plant, located in Waldwick, New Jersey, was sampled during April and May, 1978. It used a conventional and/or step aeration activated sludge system followed by disinfection with ultra-violet radiation (for this study) after which the final effluent was treated with chlorine. A block diagram of this plant is shown in Figure 4. This plant has a design flow of 32,100m^3/d (8.5 mgd). However, the total plant flow during these studies was only 19,000m^3/d (5 mgd). More details of this plant are provided by Scheible et al., 1979.

Raw wastewater entered the plant via the inlet building where all large organic and inorganic materials were removed by a bar screen, and smaller materials were cut-up by comminutors. The wastewater was then distributed through inlet and aerated channels into three primary settling tanks with an approximate capacity of 900m^3 (235,000 gal.) each. Large particles and organic matter settled to the bottom and formed a thick, raw sludge which was removed by pumps. All floatable solids were removed by mechanical skimmers.

The wastewater then flowed into two aeration tanks with a capacity of 3,300m^3 (884,000 gal.) each and was subsequently mixed with activated sludge (30% by volume). After about 6 hours of contact with diffused air, the mixed liquor flowed into final settling tanks.

The clarified secondary effluent then flowed through an ultra-violet disinfection unit manufactured by Pure Water Systems Inc., of Fairfield, New Jersey. The prototype unit consisted of a stainless steel rectangular compartment housing 400 ultraviolet lamps (85 watts input per lamp, 35 watts of UV output per lamp). The UV radiation was produced at the germicidal wavelength of 254 nm. The lamps were protected from the water by 23 mm (0.9 inch O.D. quartz jackets, which were spaced 12.7mm (0.5 inch) apart. The contact time at 3,800m^3/d (6 mgd) was approximately 3.6 sec.

After passing through the ultra-violet disinfection stage, the liquid effluent was treated with chlorine as required by the State of New Jersey before it was discharged into Ho-Ho-Kus Brook. The raw sludge, floatable solids and waste activated sludge were dewatered by centrifugation and chemical (polymer) addition, incinerated, and the ash disposed of at an off-site

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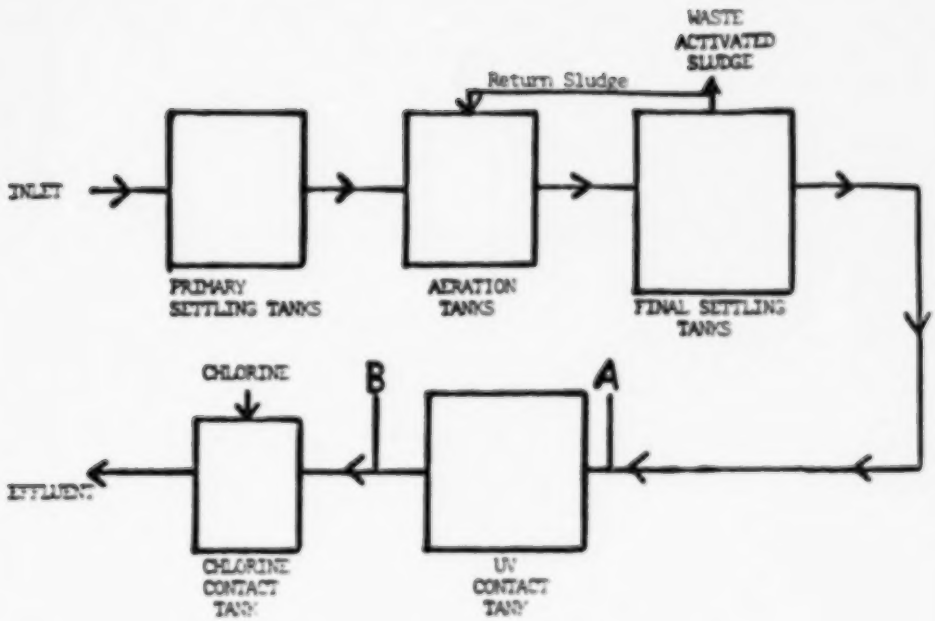

Design: Avg. Flow 32,100m^3/d (8.5 mgd)
Sludge Dewatering: Centrifugation
Disinfectant: UV light
Sampling Point A: 3.0m From UV Contact Tank
Sampling Point B: At Exit Point of UV Lights

Figure 4. Northwest Bergen County Treatment Plant (N.J.) and sampling sites.

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landfill.

Sample Point A: Secondary Effluent Sampling Site--

This source was located in a concrete basin originally used as a chlorine contact tank. For this project the basin served to transport the unchlorinated clarified liquid from the final settling tanks to the ultraviolet radiation apparatus.

The water depth in the basin was approximately 2.4m (8 ft.) deep and a submersible pump was typically located 0.6m (2 ft.) to 0.9m (3 ft.) deep in this stream.

Sample Point B: Ultraviolet Irradiated Effluent Sampling Site--

This source was located on the effluent side of the ultraviolet radiation apparatus immediately adjacent to the wiper track in the center of the effluent flow stream (0.8m below actual water surface).

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SECTION 5

EXPRIMENTAL PROCEDURES

SAMPLE COLLECTION EQUIPMENT AND VIRUS CONCENTRATOR

A Carborundum Company mobile laboratory was driven to and remained at each site during the extended sampling periods. Figure 5 is a side view of this laboratory on-site at Estes Park, Colorado. The unit was fully self-contained with onboard generators, water tanks, air conditioning, sinks, and storage facilities for chemicals and equipment.

All sample collection and treatment occurred inside this mobile laboratory by pumping water through a closed series of pumps and hoses from the sampling points into the laboratory. The internal laboratory environment was maintained to eliminate spurious inputs from the nearby waters, atmosphere, and general onlookers. Virus concentrations were generally quite low and hence required extra care in sample preparation.

Carborundum's Aquella™ Virus Concentrators were mounted inside this laboratory and used throughout the program. The Aquella™ Virus Concentrator was a portable, self-contained apparatus designed to remove water-borne viruses from large sample volumes on a continuous basis. The purpose during this phase of the experiments was to field process approximately 380 l (100 gal.) of wastewater per sample, concentrate to obtain as small a volume of liquid as practicable for analysis, and to ease handling and shipping problems.

Two units mounted on the side walls of the mobile laboratory were used for this program. Figure 6 shows one of these units bolted in place on the passenger side wall of the van shown in Figure 5.

This arrangement was somewhat different from the standard configuration in which the free-standing unit is connected together with hinges and end panels for ease of handling and shipping. Since the van was used at all wastewater plants studied, the units were permanently mounted.

Disinfection Procedures

All sample hoses, pumps, filter holders, and tubes were rinsed, disinfected, and rinsed again prior to collecting each

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Figure 5. Carborundum Company Mobile laboratory on site at Estes Park, Colorado.

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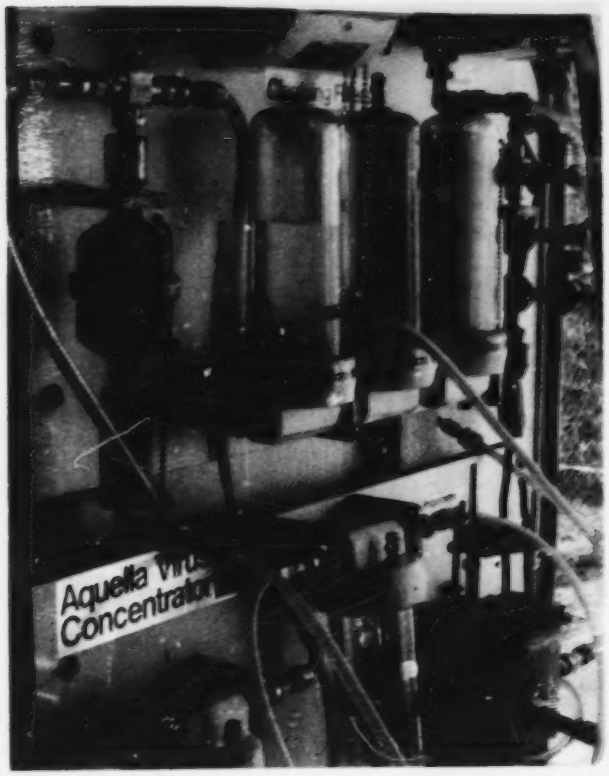

Figure 6. Virus Concentrator Wall - mounted Aquella unit in mobile laboratory.

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sample. Dilute HCl at a pH of 0.5 was allowed to sit inside all components for 30 minutes prior to the final rinsing. The disinfection procedures have proven to be very successful, especially when sampling potable waters in the vicinity of highly contaminated waters such as typically found in raw wastewater areas.

The mobile laboratory walls, ceiling, and floor were disinfected prior to collecting each sample. Chlorinated water was sprayed on all surfaces and allowed to dry. In addition to laboratory sterilization procedures, Carborundum field engineers were trained to insure personal cleanliness. This is especially important while sampling sewers, settling tanks, and aeration chambers. Every effort was made to eliminate cross contamination between the equipment, the laboratory and the on-site personnel.

Sample Collection Procedures

Secondary effluent was collected by placing a submersible pump in the center of the flow stream being sampled. Water was pumped through one of several nylon garden hoses to the inlet of the Aquella™ Virus Concentrator shown at the right of Figure 6. A flow rate of 0.06 l/s (1 gpm) was typical. The water flowed through an impeller pump and then through three clarifier filter holders, which may or may not contain clarifier filters depending on the suspended solids concentration in the wastewater. Several times during these experiments, clarifier filters were required. Once through the clarifiers, water flowed through a chemical proportioning pump which had several satellite liquid injectors. Conditioning chemicals were added at predetermined rates, which were automatically controlled by volume flow. This feature becomes important, as filters plug and flow rates correspondingly decrease, to ensure proper final concentrations of conditioning chemicals in the test waters.

Sodium thiosulfate, dilute HCl, and $AlCl_3$ were added through the satellite liquid injector chemical proportioning pumps. The exact concentrations were dependent upon the wastewater characteristics, but our researchers and consultants have shown optimum virus recoveries for a pH adjusted between 3 and 4, $AlCl_3$ concentrations near 1.5 mM, and sufficient sodium thiosulfate to eliminate active chlorine, ozone or other oxidizing compounds.

A flow totalizer was mounted in the flow stream just prior to the two custom designed virus adsorbing filters shown on the lower left of Figure 6. After passing through these two final filters, the wastewater was returned to the treatment plant.

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Virus Elution and Concentration Procedures

Most viruses are collected on the two final adsorbing filters. However, clarifying filters, when they are used, must also be subjected to the virus elution procedures to account for viruses trapped in solids.

Viruses were removed from the filter surfaces using controlled methodologies which have been developed over several years at he Carborundum Company as well as at other research and governmental laboratories. These procedures included a saline rinse to remove residual chemicals, and virus elution using 0.5 M glycine at a pH above 10. This step typically produced 1 to 3 l of solution.

Further liquid volume reduction was accomplished in the field using a gel reconcentration technique which incorporates 3 mM $AlCl_3$ coupled with Na_2CO_3 to form a floc at pH 7. Floc was separated from the mixture either by gravity or centrifugation.

Viruses were removed from the floc by repetitive, vigorous agitation as the pH was raised above 10 with 0.5 M glycine (pH 10.5). The resulting supernatant was collected and neutralized to pH 7.4 with 0.5 M glycine (pH 2.0) before the addition of 10% by volume fetal calf serum. This final concentrate was quickly frozen at dry ice temperatures and shipped to the assay laboratory. Shipping was in accordance with all Federal, State, and Carrier regulations.

Enterovirus Seed Experimental Procedures

Typically, two 208 l (55 gal.) Nalgene containers, each connected to a virus concentrator, were filled in the field with 190 l (50 gal.) of the test effluent. Sodium thiosulfate was added to each container at a concentration equivalent to quench 10 mg/l free chlorine. The seed virus was prepared using Hank's Balanced Salt Solution (BSS) plus 10% fetal calf serum to form a dilute mixture. One ml of this diluted stock was placed in approximately 4 l of the test effluent taken from one of the 55 gal. drums.

After mixing, these 4 l were returned to the same drum. Thus, the total volume of seed solution was approximately 190 l (50 gal.). The second sample of test effluent was not seeded, and served as a background virus control. Both were sampled independently, but simultaneously. Both low and medium titer runs were performed at each site. The low and medium titer seed experiments were identical in design with the following exception:

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1. There was a quantitative difference in input virus titer between the low and medium titer runs. Titer was determined by quality of water being sampled and expected viability of input seed virus. Low titers were between 6.8×10^1 and 6.2×10^3 plaque forming units (pfu) per 190 l (50 gal.) sample, and medium titers between 2.0×10^3 and 4.0×10^4 pfu's per 190 l (50 gal.) sample.

2. All low titer runs were performed on wastewater effluent after disinfection and all medium titer runs on effluent prior to disinfection.

The virus seed specifications used at each test site and the disinfectant used were as follows:

1. Muddy Creek Works Effluent at Taft Center Pilot Plant; Carborundum Lot No. 7 Type 1 Poliovirus; ozonation.
2. Muddy Creek Works; Carborundum Lot No. 12B Type 1 Poliovirus; chlorination.
3. UTSD Treatment Plant; Carborundum Lot No. 11 Type 1 Poliovirus; ozonation.
4. Marlborough Easterly Wastewater Treatment Plant; Carborundum Lot No. 13 Type 1 Poliovirus; chlorination.
5. Northwest Bergen County Treatment Plant; UNH Lot May, 1978 Polio LSC - 1 (Type 1 Poliovirus); ultraviolet radiation.

During these experiments, control samples were taken for possible assay in addition to the final eluates and background virus samples. Typically, a toxicity control sample was collected by removing 1000 ml of the test effluent sample from the 190 l Nalgene™ drum prior to seeding. One ml of a 1:10 dilution of seed stock was added to this 1000 ml sample, after mixing and prior to the start of the experiment. One ml of this toxicity control sample was then diluted 1:10 in BSS. Again, at the end of the experiment 1 ml of the original 1000 ml toxicity control was diluted 1:10 in BSS medium. The remainder of the unused seed stock from four of the P.O.T.W.'s was shipped to the assay laboratory. Since all seed stock was utilized at Marlborough Easterly, the remainder of the input seed virus dilution was forwarded. Finally, a dilution control was prepared by adding 1 ml of the seed dilution used in the experiment to a volume of BSS + 10% fetal calf serum equal to the volume of the final eluate concentrate.

Stock seed virus concentration calculations--

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A stock virus seed control was typically prepared by placing 1.0 ml of 1:10 dilution seed into 1000 ml of test effluent. A 1.0 ml aliquot of this 1000 ml virus/effluent mixture was placed in 9 ml of BSS + fetal calf serum.

R_S = number of viruses recovered (pfu) from control solution inoculum.

V_I = volume of seed virus + BSS + 10% fetal calf serum; quantity inoculated at assay laboratory (typically 0.1 ml)

V_D = specific dilution plaqued.

The total seed virus concentration possible in the input water (ie., in the 4000 ml which was added to the 190 l (50 gal.) effluent sample was:

C_v (total virus concentration in pfu/ml) = $R_S \div (V_I\ V_D)$

Stock seed virus, typically 1.0 ml, was added to a measured volume (4000 ml) of neutralized distilled water and added to the 190 l (50 gal.) Nalgene™ drum.

V_s = volume of stock virus in Nalgene™ drum (1.0 ml).

f = fraction of drum solution through unit during seed run (typically 1.0%).

The total seed virus which passed through the Aquella™ and into the sample water was:

C_s = (total virus concentration through the unit (pfu)) = $C_v \times V_s \times f$

Virus recovery calculations--

The 190 l (50 gal.) seed run was processed (eluted and concentrated) and a volume of concentrated frozen fluid containing virus was shipped to the assay laboratory. The laboratory further reduced this volume (except the Northwest Bergen County sample) and assayed the new concentrate. It reported the number of viruses recovered from a known volume of the concentrate plaqued from a specific dilution. Let:

V_A = total volume of concentrate for analysis

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R_C = number of viruses recovered (pfu) from concentrate inoculum

V_C = volume of inoculum plaqued

V_p = specific dilution plaqued

The concentration of the viruses recovered from a seed run was computed as follows:

C_C (virus concentration (pfu/ml)) $= R_C \div (V_C\ V_p)$

The total number of viruses detected in the seed run was:

C_R (total number of viruses detected in seed run (pfu)) $= C_C \times V_A$

B = background viruses from simultaneous run

$$\% \text{ Recovery} = \frac{\text{No. pfu detected}}{\text{No. pfu input}} = \frac{C_R - B}{C_S}$$

LABORATORY TECHNIQUES

Samples collected during this program were analyzed for virus numbers and identification in the Virus Laboratory, University of New Hampshire. Dr. T. G. Metcalf (Carborundum Company Consultant) was the coordinator of this phase of the program. A schematic diagram (Figure 7) depicts the procedure followed for each sample.

Each sample was maintained at -80°C until placed on test to avoid denaturation and other changes detrimental to virus recovery and infectivity. Removal of non-viral contaminants was accomplished by treatment of samples with antibiotics (1 hour at 26°C) or diethyl ether (12 - 16 hours at 5°C). The antibiotic mixture contained 100 units of penicillin, 100 ug streptomycin, 3 ug fungizone and 25 ug gentomycin sulfate as final concentration per ml. Samples treated with ether had a final ether concentration of 20 percent (v/v).

Samples greatly in excess of 30 ml volume required further reduction of volume before they could be analyzed for virus. Such samples were placed in dialysis tubing and the tubing placed in contact with polyethylene glycol 20000 at 5°C.

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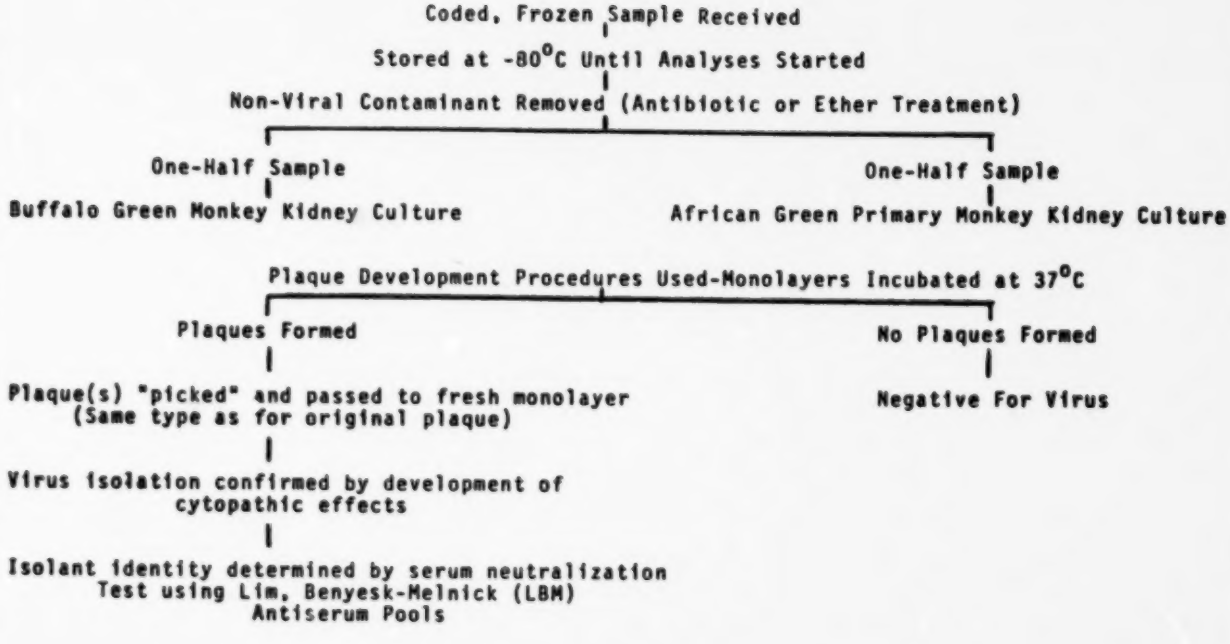
Coded, Frozen Sample Received
Stored at -80°C Until Analyses Started
Non-Viral Contaminant Removed (Antibiotic or Ether Treatment)
One-Half Sample
Buffalo Green Monkey Kidney Culture
One-Half Sample
African Green Primary Monkey Kidney Culture
Plaque Development Procedures Used-Monolayers Incubated at 37°C
Plaques Formed
No Plaques Formed
Plaque(s) "picked" and passed to fresh monolayer
(Same type as for original plaque)
Negative For Virus
Virus isolation confirmed by development of
cytopathic effects
Isolant identity determined by serum neutralization
Test using Lim, Benyesk-Melnick (LBM)
Antiserum Pools

Figure 7

Test Procedures Used for Virus Analyses of Test Samples

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Dehydration of the sample followed within a few hours, resulting in a reduction in volume to 15 to 20 ml. The dialysis tubing was rinsed with phosphate buffered saline, pH 9.0, containing 2% fetal calf serum. The washing was added to the dehydrated samples, giving a final volume varying from 22 to 34 ml. The above procedure is called hydroextraction.

Analysis of samples for natural virus was carried out on a continuous type of cell culture (Buffalo Green Monkey - BGM) maintained in the UNH Virus Laboratory, and a primary type of cell culture (African Green Monkey Kidney-PMK) prepared fresh for each sample from suspensions of kidney cells obtained from Microbiological Associates, Walkersville, Md. The two types of cell cultures were used in order to obtain an isolation sensitivity which presumably was greater than the sensitivity which would have been obtained through the use of only one type, regardless of whether it was the BGM or the PMK culture.

Assays of seed virus samples (stock poliovirus) were made using the BGM culture since the poliovirus had been propagated on this culture and it (BGM culture) was of maximum sensitivity for assay purposes.

The virus assays in cell cultures were made by plaquing methods which permitted separation and enumeration of individual viruses present in samples. Assays were carried out in either 9 cm^2 monolayer (1 oz.) or 45 cm^2 monolayer (6 oz.) bottles. Only one-half of the sample allocated per cell culture was tested at one time. The other half was maintained at -80°C and tested at a later date. This strategy was adopted to avoid the loss of an entire sample if sample toxicity for the cell cultures was shown.

Inocula added to cell cultures (0.1 ml per 9 cm^2 monolayer and 1 ml per 45 cm^2 monolayer) were allowed to adsorb for 1 hour at 37°C, followed by removal of the inocula and a gentle rinsing of the monolayer using phosphate buffered saline. The monolayers were overlaid with an agar overlay medium containing (per 100 ml): penicillin (10,000 units), streptomycin (10,000 ug), 1.5 grams Difco agar, 40 ml concentrated Minimal Essential Medium, 1 ml each of concentrated L-glutamine, concentrated nonessential amino acids, 1 gram $MgCl_2$, and 1 ml Sterile Milk (Real Fresh Inc., Visalia, CA.), 2 ml heat-inactivated fetal calf serum, 3 ml 7.5 grams $NaHCO_3$, and 0.6 ml diluted neutral red. Monolayers were incubated at 37°C until plaques appeared or deterioration of the monolayer occurred (usually about 20 days).

Upon development of a plaque, it was "picked" and transferred to a fresh cell culture of the same type yielding the initial plaque. The culture was overlaid with a liquid main-

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tenance medium and observed for development of cytopathic effects. Development of cytopathic effect in the absence of non-viral contaminants was confirmation of the virus etiology of the plaque.

Natural virus isolates were identified by means of serum neutralization tests using eight antiserum pools - Lim-Benyesh-Melnick (LBM pools). The tests were carried out in microtiter plates using 0.025 ml volumes of virus (100 pfu) and antiserum pools (10 Antibody Units). Identification of an isolate was determined by the pattern of neutralization of virus cytopathic effects in the eight serum pool test.

Analyses for natural virus made use of two separate laboratories and special facilities within each laboratory to reduce to an absolute minimum any chance for laboratory contamination of test samples. An outline of the procedures used and test policy followed is given in Table 1.

The UNH Virus Laboratory located in the Jackson Estuarine Laboratory was specially designed for contamination-free, natural virus studies. The laboratory consists of an inner room with two Baker Sterilgard cabinets. The room is completely separated from an access corridor. Inoculation of monolayers at the Jackson Laboratory was carried out in an environment in which no stock viruses had ever been introduced. This avoided any chance for cross contamination of the monolayer from a laboratory stock virus source. Return of all plaque-containing monolayers to the campus laboratory for plaque "picks" eliminated any chance for introduction of natural virus into the Jackson Laboratory movement. The execution of the "picking" process within a Sterilgard cabinet in a campus laboratory in which no stock virus had been knowingly introduced (i.e. for experimental or assay purposes) avoided any known opportunity for cross contamination of monolayers or "picks". The phrase "knowingly introduced" refers to the fact that while virus experiments using stock viruses were being conducted in other virus laboratories on the same floor of the building within the Department of Microbiology, the laboratory in which the "picks" were made was physically separated and not used for experiments or assays.

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TABLE 1. THE COMBINED USE OF SEPARATE LABORATORIES FOR ANALYSIS OF SAMPLES FOR NATURAL VIRUS

Test Procedures	Virus Laboratory	
	*Campus	**JEL
1. Sample received, stored at -80°C	+	
2. Additional treatment i.e. concntration, removal of non-viral contaminants		+
3. Cell culture tests		+
4. Plaque "picks"***	+	
5. Virus isolate confirmed	+	
6. Identification of isolate	+	
7. Storage of isolate	+	

* Located within Department of Microbiology, University of New Hampshire.

** Jackson Estuarine Laboratory, 6 miles distant from campus.

***Carried out within Baker Sterilgard Cabinet.

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Plaques: Plaque-Forming Units (Pfu's)

A plaque is a visual manifestation, usually on a microscopic scale, of a region of cells whose vitality has been destroyed or reduced as the result of a localized influence. The area is demonstrated by the failure of a vital stain (one which interacts with and colors only viable cells) to produce the typical color in an area demonstrating a cytopathic effect. Any influence which leads to a cell necrosis will result in either the alteration or elimination of vital stain, thus delineating the area clearly as a region of different color, or by definition, a plaque.

A plaque is the result of the effects of a plaque forming unit of virus or other agent. A plaque forming unit is not necessarily equivalent to a single virus on a numerical basis. The number of pfu's is always less than or equal to the actual number of viruses. For example, a clump, or aggregate of 10, 100 or even more virus particles (virions) may attach to localized cells in a monolayer and produce a single plaque.

Depending on the susceptibility of a cell type toward virus infection or the tendencey of a virus to form a plaque, on the age of the tissue culture and on other factors, the efficiency of a virus to absorb, penetrate, replicate and develop plaques is much less than unity. Certain cell types may be several orders of magnitude less efficient than others toward specific virus types, or even strains of the same virus. According to the American Type Culture Collection (ATCC) index, Poliovirus 2 forms plaques 1000 times more efficiently on PMK cells than on one strain of HEP-2 cells. Other viruses, such as the reoviruses, may be several orders of magnitude less efficient in plaque forming capability than they are by CPE (cytopathic effect) using liquid overlay techniques. We have chosen the best compromise for this program.

Figures 8 and 9 present a series of photographs which represent typical plaques of Polio, Echo, and Coxsackie virus. These plaques are then "picked" wherein the virus to be identified is transferred to a fresh culture monolayer with a liquid overlay medium. Identification of isolates is then carried out in microtiter plates using the Lim-Benyesh-Melnick antisera pools. Identification is hindered, however, when more than one type of virus is involved in a pfu (termed mixture) and in some cases, identification becomes impossible (termed unidentified) due to toxicity, non-reactivity, or non-culturability.

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Figure 8. Typical appearance of poliovirus plaques.

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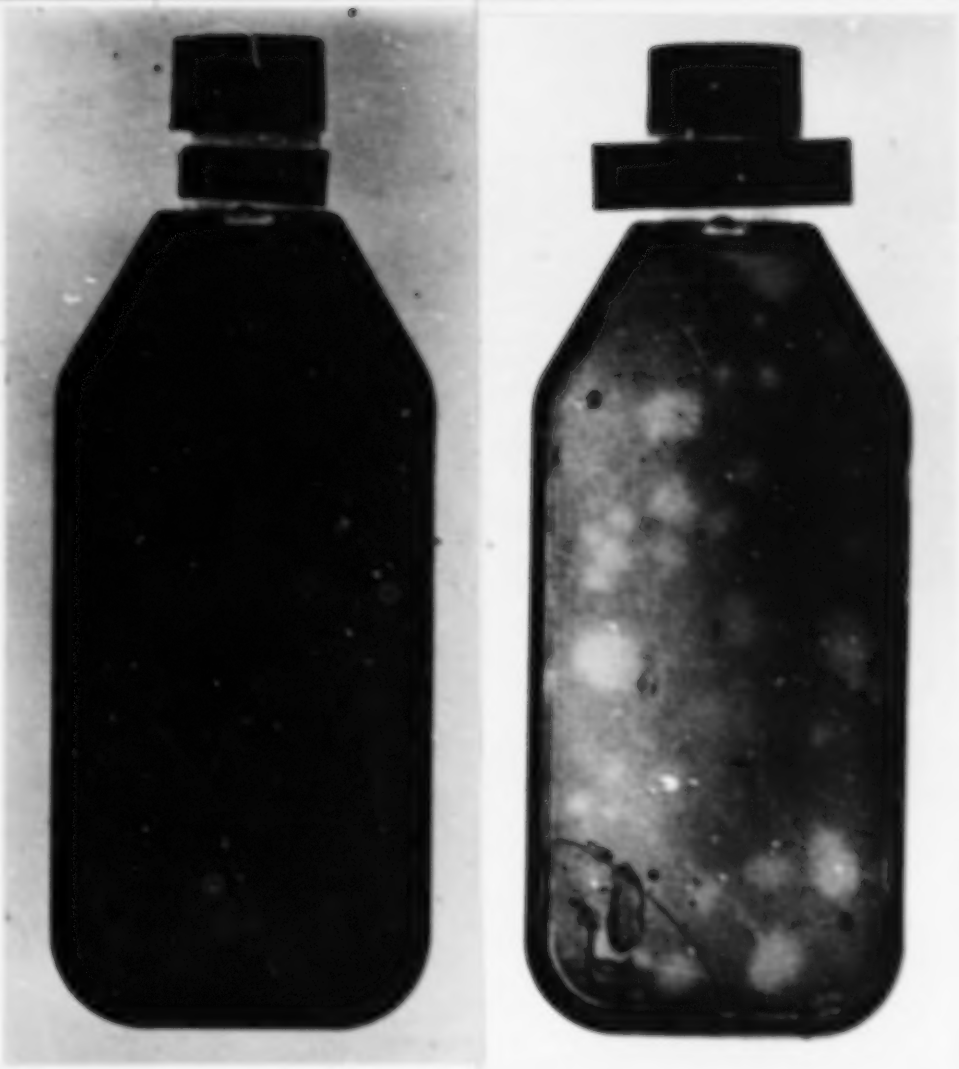

ECHO COXSACKLE

Figure 9. Typical appearance of Echo virus and Coxsackie virus plaques.

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Water Parameters and Coliform Data

All physical-chemical and bacteriological data were provided by EPA or wastewater treatment plant personnel. All parameters were measured on the days of virus sampling and the samples were analyzed according to accepted standard methodologies for water and wastewater (Standard Methods - 14th Ed., 1975). Samples collected at the wastewater treatment plants were usually analyzed for:

total and fecal coliforms - MPN; total COD - mg/l; soluble COD - mg/l; TOC - mg/l; Temp - ^{o}C: Turb - JTU; TSS - mg/l; VSS - mg/l; TKN - mg/l; NH_3-N-mg/l; NO_3-N-mg/l; NO_2-N-mg/l; Fe - mg/l; Mn - mg/l; Total Cl_2 Res. - mg/l; Ozone (O_3) residual - mg/l; U.V. Abs.; Color - PCU

Linear regression analyses and Student's t tests were performed on the pertinent data in an attempt to determine which indicators and parameters would correlation with the virus enumeration data.

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SECTION 6

RESULTS

VIRAL CONTENT IN P.O.T.W. EFFLUENTS

These results represent the cumulative data for the effluent samples (pre and post disinfection) from the four treatment facilities. Assay results of all natural enterovirus sample runs are presented in tabular form in Appendix A.

Virus Isolation Rates and Concentrations

Virus isolation rates are individually reported and presented as a histogram for each of the four wastewater treatment plants in Figure 10.

The width of each rectangle represents the test period and the height represents the average isolation rate. These isolation rates were calculated for both the treated and treated plus disinfected effluent. The rates were determined by equally weighting each sample which contained one or more virions. The data from each sample which contained one or more virions were then tabulated to form the reported average. Based on this weighting, 38 percent of all samplings, prior to disinfection, contained one or more virions. The actual virus isolation rates for the wastewater effluent prior to disinfection from each facility were: UTSD Treatment Plant, 69%; Muddy Creek Works, 75%; Taft Pilot Plant, 57%; Marlborough-Easterly Plant, 38%; Northwest Bergen County Plant, 56%.

These results showed no major differences between seasonal isolation rates, particularly for the two sampling periods completed in July and December at the Muddy Creek Works. The latter two rates for non-disinfected effuent were 75% and 60% respectively, and determination of a significant difference would require more testing.

The darkened area in Figure 10 shows the virus isolation rate after the disinfection step. The virus isolation rates depicted show that significantly lower isolation rates were encountered after disinfection. Reductions occurred for each of the three disinfectants (UV, chlorine, and ozone) studied. The actual virus isolation rates for the wastewater effluent after disinfection from each facility were: UTSD Treatment Plant (all sample days), 50%; UTSD (eliminating four days of

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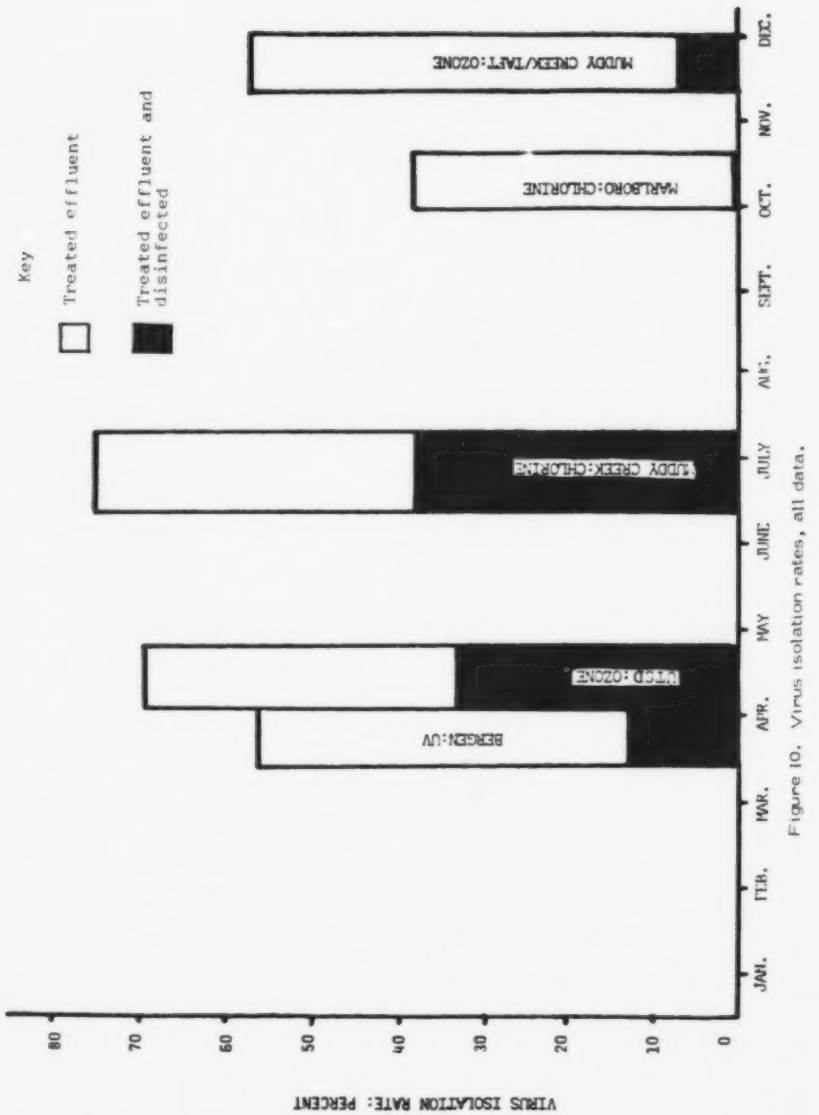

Figure 10. Virus isolation rates, all data.

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deliberately decreased ozone dosage), 33%; Muddy Creek Works, 38%; Taft Pilot Plant, 7%; Marlborough-Easterly Plant, 0%; Northwest Bergen County Plant, 13%.

Virus isolation rates were reduced 75 percent when ultraviolet light was used as a disinfectant; 50 percent when ozone was used at UTSD; 88 percent when ozone was used for Muddy Creek at Taft; and 50 percent and 100 percent (none detected) for the two chlorine samplings.

These data were also analyzed to obtain an average virus concentration per 380 l (100 gal.) of both treated and treated plus disinfected effluent. All samples taken at each plant were analyzed and the individual plant results were averaged. The results represent the average concentrations for approximately 11,370 l (3,000 gal.) of effluent collected at each site over the entire test period. Figure 11 is a comparison of these average concentrations.

These results again indicate no significant seasonal differences, especially when comparing the two Muddy Creek Works data, before disinfection, for July and December. Both sets of data show about six virions per 380 l (100 gal.) of treated effluent.

The UTSD Treatment Plant had the poorest average ratio of disinfected to treated effluent virus content of those plants measured, if one considers all of the data collected. This ratio, calculated from Appendix A data, would only be 0.7. However, during four days of the test period, the applied ozone dosage was deliberately decreased to less than 3.0 mg/l. Hence, this was likely the cause of the increased virus levels in the ozonated effluent. Neglecting the data collected on those four days results in an average ratio of disinfected to treated effluent virus content of 0.04, as shown in Figure 11. Variation in virus levels as a function of the ozone dosage will be presented later (see Figure 18).

Statistical Significance of Disinfection Data

Application of the Student's t-test to all of the measured virus isolation rates and concentrations is given in Table 2.

The t-statistic is given along with its significance level. The smaller the significance level, the more likely the two virion distributions (before and after disinfection) are significantly different. A low (below 5%) significance level is an indication that the two means are significantly different, i.e., that the decreases are due to a controlled variable, such as disinfection in this case.

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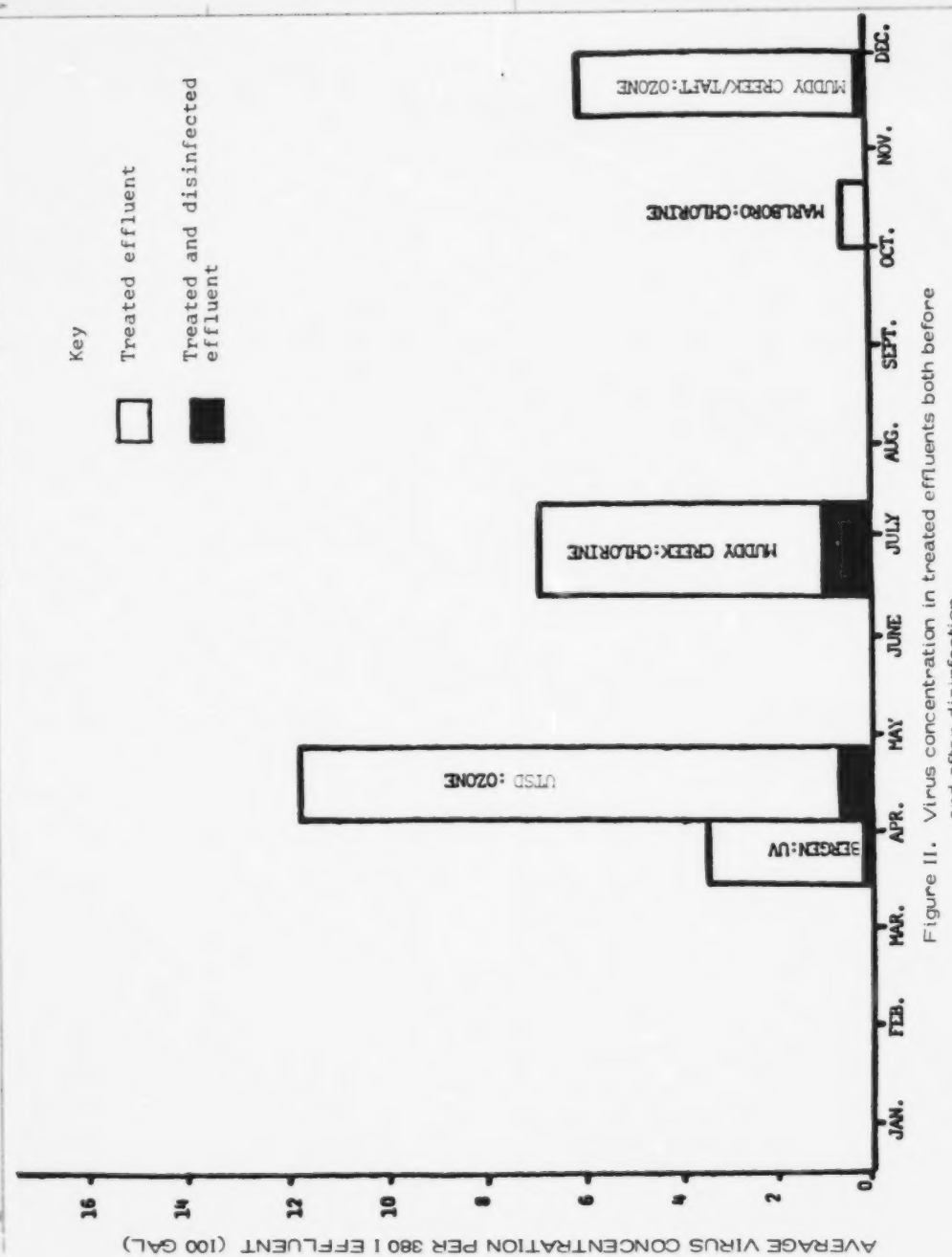

Figure 11. Virus concentration in treated effluents both before and after disinfection.

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TABLE 2. STUDENT'S t SIGNIFICANCE TEST FOR VIRUS IN TREATED AND DISINFECTED EFFLUENT

	Average disinfected effluent titer, virions per 380 liters	Mean of difference for treated effluent before and after disinfection, virons per 380 liters	Standard deviation Virions per 380 liters	t - Statistic	Significance level
Bergen (UV)	0.1	1.1	1.8	2.5	0.022
	(0.4)	(3.7)	(8)	(1.4)*	(0.16)
UTSD (ozone)	8.0	3.6	14	1.0	0.16
Muddy Creek (chlorine)	1.0	6.1	11	2.2	0.024
Marlborough (chlorine)	0	0.6	0.9	2.5	0.022
Muddy Creek/Taft (ozone)	0.07	6.1	12	1.9	0.039

*Bracketed values neglecting four problem days

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The average effluent concentrations and the mean value of the differences in virus titers before and after disinfection are given in Table 2. The disinfected effluent titers ranged from zero for chlorinated, nitrified effluent to 1.0 virion per 380 l (100 gal.) for chlorinated secondary effluent. A measure of virus inactivation is obtained by dividing the average discharge titer by the input titer (sum of average discharge titer and mean of the difference). These relative values range from 14% for chlorinated secondary effluent to near 0% for chlorine added to nitrified effluent (Marlborough). It should be noted that the Marlborough treatment facility employed a two-stage biological treatment procedure for nitrification and a high quality treatment procedure utilizing a turbine mixer to ensure efficient chlorine contact in the initial rapid mix. These may be the primary reasons that the pre-disinfected samples had low virus counts and the disinfected samples had no detectable virus counts.

Virus Identification

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A summary of all samplings in this program to determine the distribution of identified viruses before and after disinfection is shown in Figure 12.

The relative distribution of viruses by classification, based on the total number of viruses in all non-disinfected effluents, is presented. The polio and coxsackievirus types were separated and individually identified. All three types of polioviruses (1,2, and 3) were shown to be present in the effluents. These viruses were not assayed to determine their virulence. The wild strain of poliovirus is known to produce meningitis and paralytic polio in man. Coxsackie A9, B1, B2, B3, B4, B5, and B6 were also isolated and identified during this program. These latter viruses cause summer rash, polio-like paralysis, chest pains and colds. Note that, except for the CB5 virus, all virus types were found in the treated as well as the treated plus disinfected effluent.

Diurnal Variations

This study has shown that virus levels in treated effluent vary diurnally. Muddy Creek Works was sampled to study these diurnal variations. Virus concentrations were greater (as high as 40 viruses per 380 l (100 gal.) between 2:00 AM and 4:00 AM in both December and July, as shown in Figures 13 and 14. An increase of almost an order of magnitude is evident for virus titers between 2:00 AM and 4:00 AM. This increase remains unexplained at this time.

The increased virus input to the plant between 2:00 AM and 4:00 AM did not result in a significant increase in the disin-

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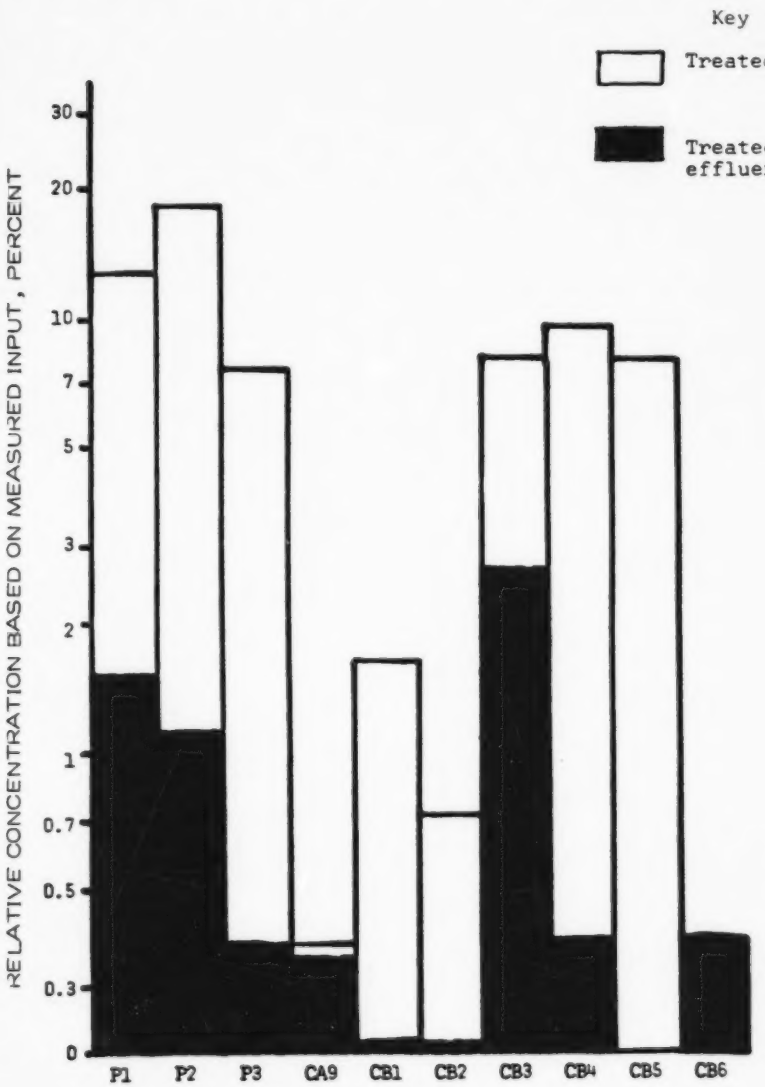

Figure 12. Distribution of virus by classification before and after disinfection with chlorine, ozone, or UV light.

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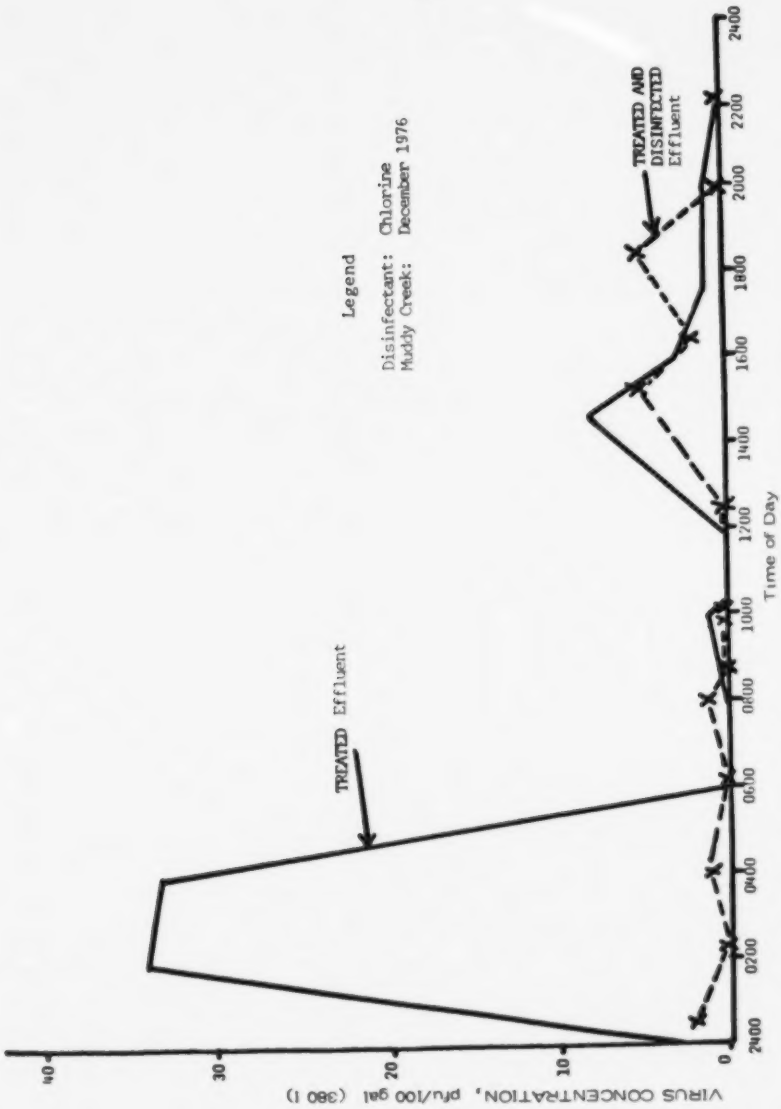

Figure 13. Diurnal variation of virus concentration in effluents: Winter

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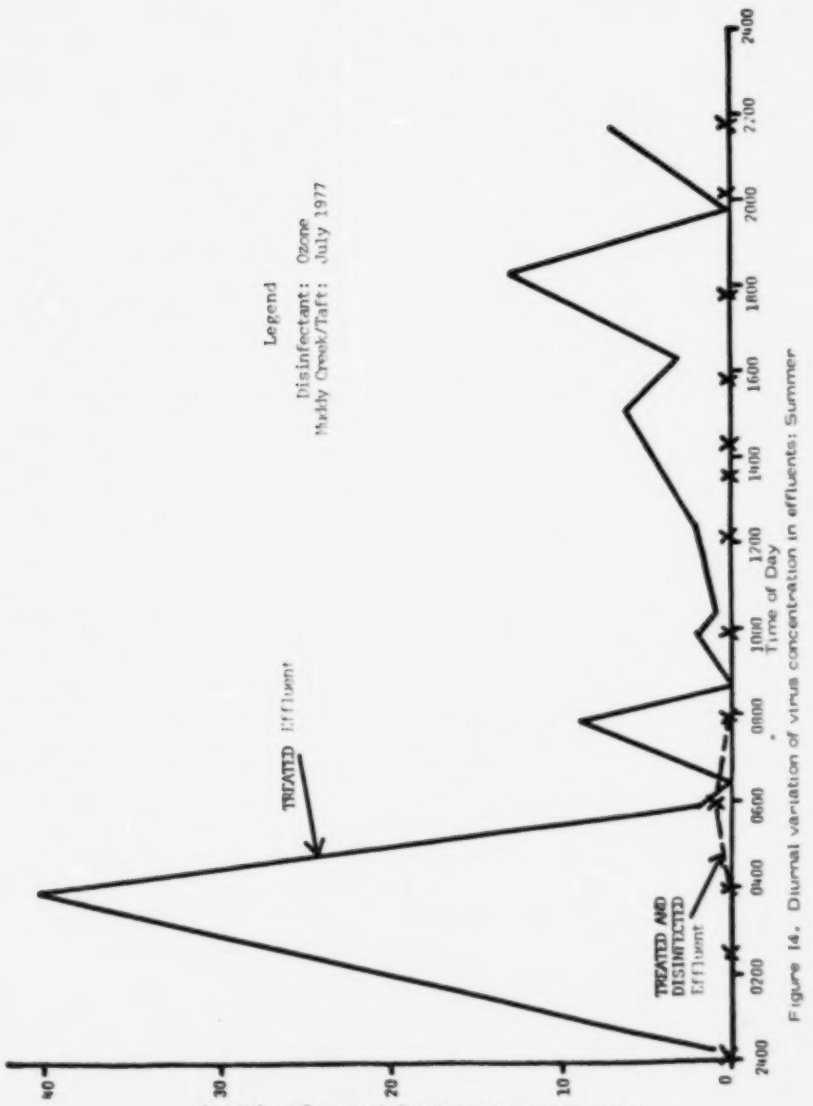

Figure 14. Diurnal variation of virus concentration in effluents: Summer

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fected effluent virus concentrations. The dashed lines indicate virus concentrations for treated, disinfected effluent. No valid comparison is available for other plants since this is the only plant visited where staggered sampling was accessable for all characteristic water quality measurements.

Virus Indicators

The dirurnal variations demonstrated at the Muddy Creek Treatment Plant provide a unique opportunity for comparisons with the traditionally measured water quality variables or hypothesized viral indicators. Appendix C provides the operating characteristics and effluent quality characteristics for all of the treatment plants.

Linear regression analyses to determine indicators that might correlate with virus levels are shown in Figure 15 for the Muddy Creek treated effluent (July data).

Total Coliform, fecal coliform, chemical oxygen demand, and total organic carbon have a positive correlation coefficient. No correlation was evident for total suspended solids and turbidity. Confidence levels, determined by t test, are shown in Figure 15 for these correlation coefficients.

A similar analysis of effluent quality data collected in December is shown in Figure 16. In this instance, no correlation was found with total coliforms and fecal coliforms while a positive correlation with total suspended solids and turbidity was observed. Both COD and TOC exhibited a positive correlation with virus titers in December as well as July.

Coliform Results

A comparison of total and fecal coliforms at each plant site visited during the two year study is shown in Figure 17. Measured total and fecal coliform numbers in the treated effluents ranged from 10^4/100 ml (Marlborough) to almost 10^6/100 ml (UTSD).

As with the virus data, there is no indication that in the summer months the effluents had higher coliform densities than other times of the year. In particular, the Muddy Creek data show a slight increase in total and fecal coliform counts in December as compared to July.

The darkened fraction of the histograms is the coliform count after disinfection.

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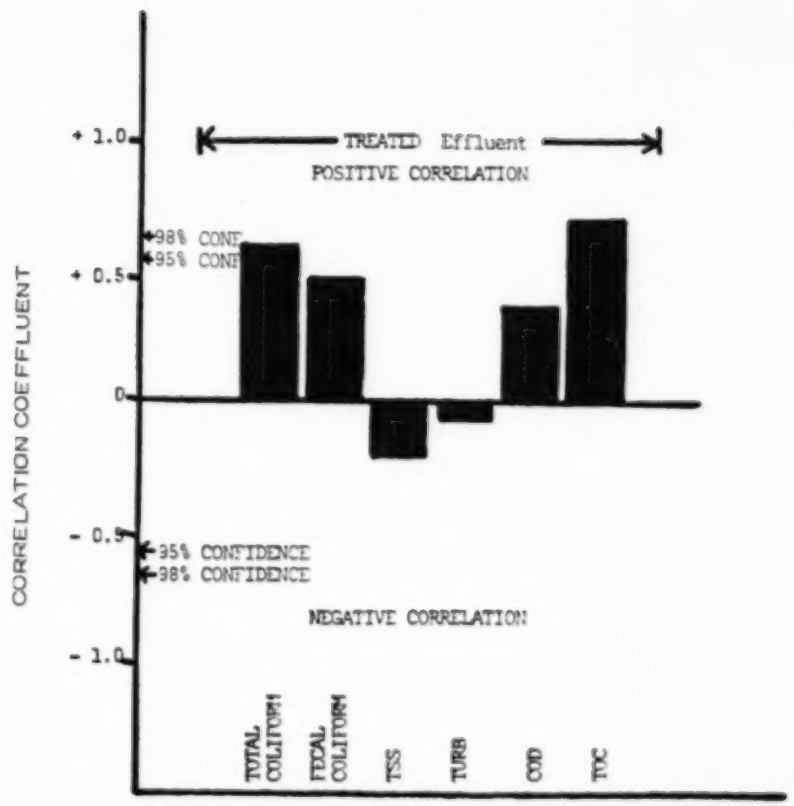

Figure 15. Correlation between virus levels in the Muddy Creek treated effluent and effluent quality characteristics (July).

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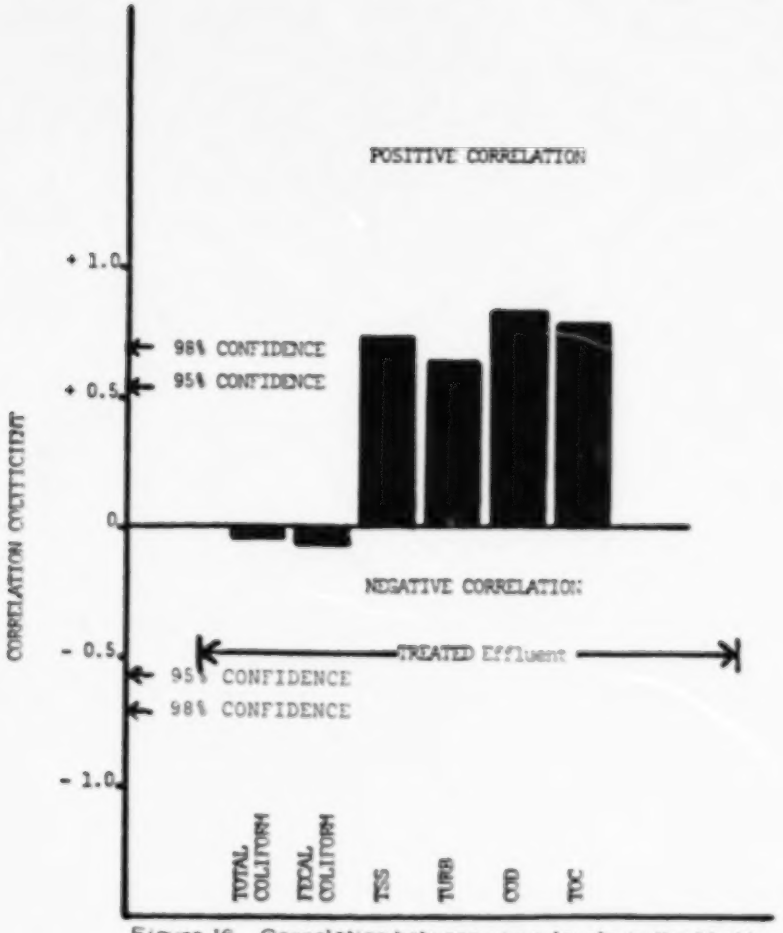

Figure 16. Correlation between virus levels in the Muddy Creek treated effluent and effluent quality characteristic. (December)

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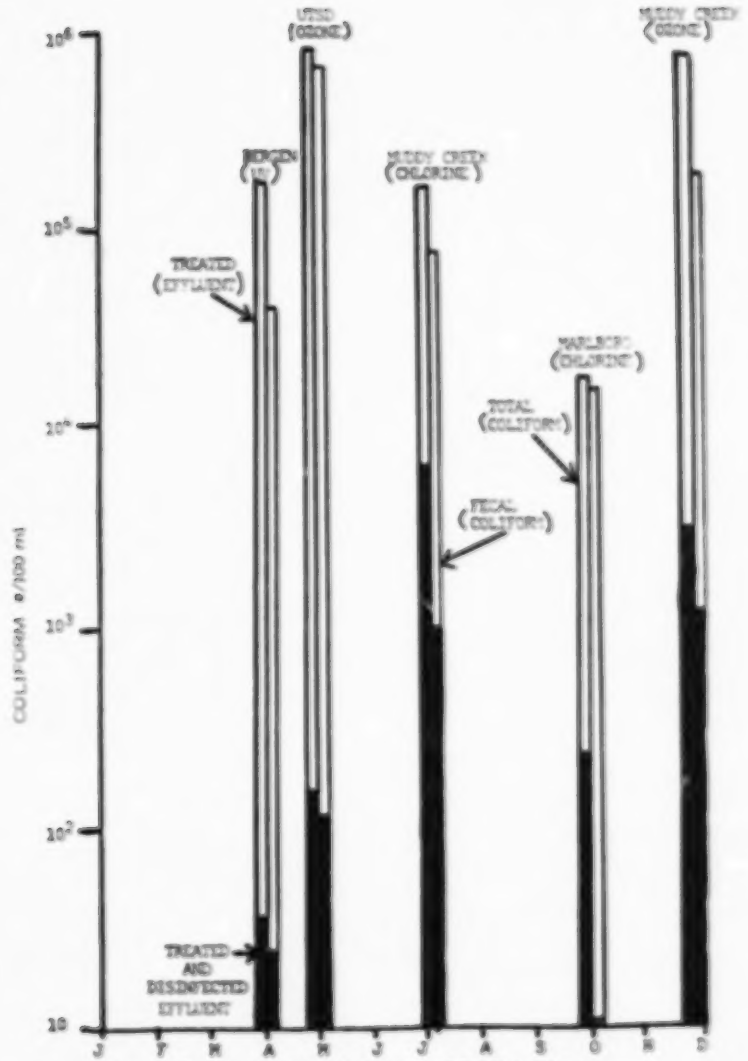

Figure 17. Monthly total and fecal coliform numbers.

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Ozone Disinfection Results

As previously indicated, the ozone dosage was deliberately reduced at the UTSD Treatment Plant during 4 days of the 15 day period to obtain a dose response curve. Figure 18 shows virus data and Figure 19 shows total coliform data as a function of applied ozone dosage.

The correlation coefficient for virus die-off as a function of ozone dose ($r = 0.67$) was calcualted by neglecting the less than one virus values measured at higher dosage levels. This was necessary since a semi-log plot of the virus titer as a function of dose requires non-zero entries for classical rate equation analysis.

The linear regression model for total coliforms indicates that an applied ozone dose of greater than 4.5 mg/l would be required to lower the total coliform count below 100/100 ml.

VIRAL CONTENT IN ENTEROVIRUS SEED EXPERIMENTS

The results of the enterovirus seed runs are presented in tabular form for each treatment plant in Appendix B. The seed experiments were performed as a control technique and to measure the efficiency of the Aquella™ virus recovery system.

Two enterovirus seed runs were performed at each of the four plant sites. In all instances, a low titer run was conducted on the effluent after disinfection, and a medium titer run on the effluent prior to disinfection (higher titer to avoid masking of the seed viruses by naturally occurring viruses). Recovery percentages at UTSD (7.7%), Muddy Creek (46.2%) and Northwest Bergen County (68.0%) for the low titer runs and recovery percentage at UTSD (9.9%), Northwest Bergen County (14.5%), Taft (2.3%), and Muddy Creek (1.6%) for the medium titer seed runs reflect real-world differences in changing water quality, water toxicity, and equipment recovery efficiencies.

In the final seed runs performed for this project (Northwest Bergen County), a special UNH monodispersed polio LSC-1 virus culture (Lot May, 1978) was used as the stock line. This line of virus seed stock and its descendants supplanted all other seed stock previously employed and results in uniformly improved recoveries. The seed virus recovery percentages at Northwest Bergen County were the highest of all the plants tested for both the low (68.0%) and medium titers (14.5%). These findings may indicate that the UNH seed stock had a greater vitality and uniformity of titer than the previously used seed stock lines.

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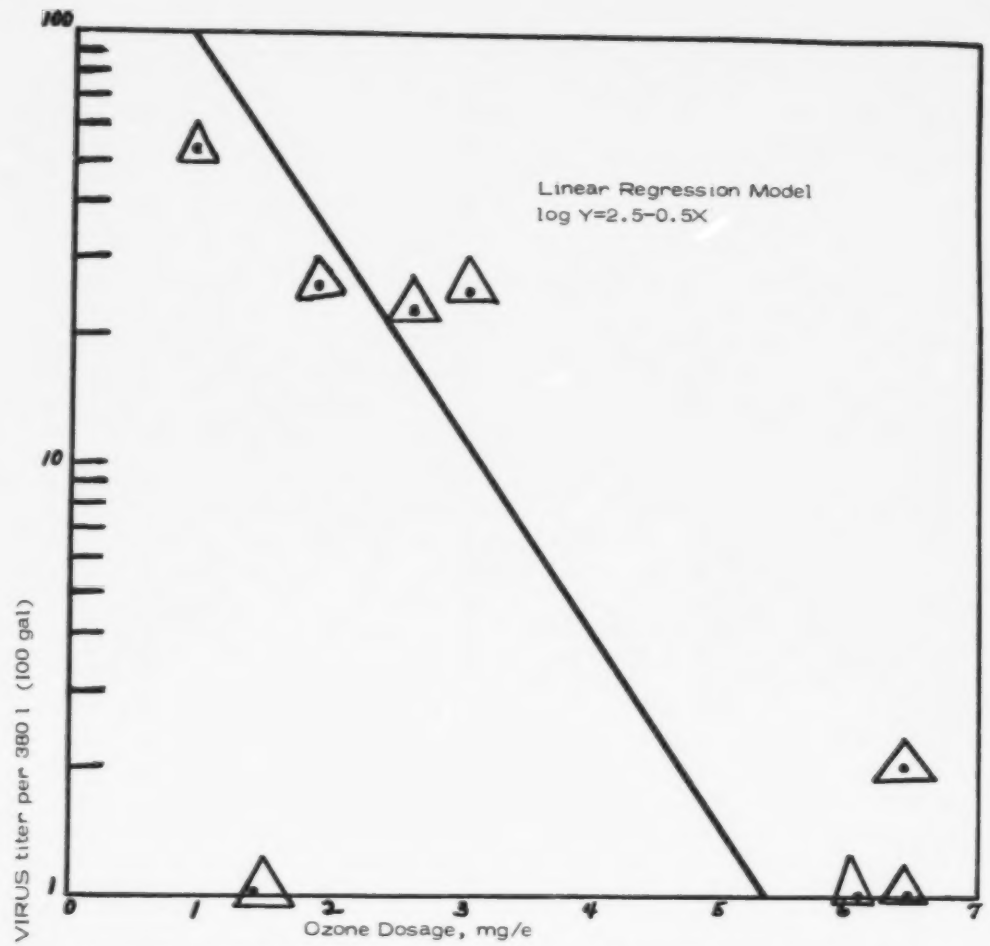

Figure 18. VIRUS TITER AS A FUNCTION OF APPLIED OZONE DOSAGE UTSD (MAY, 1977).

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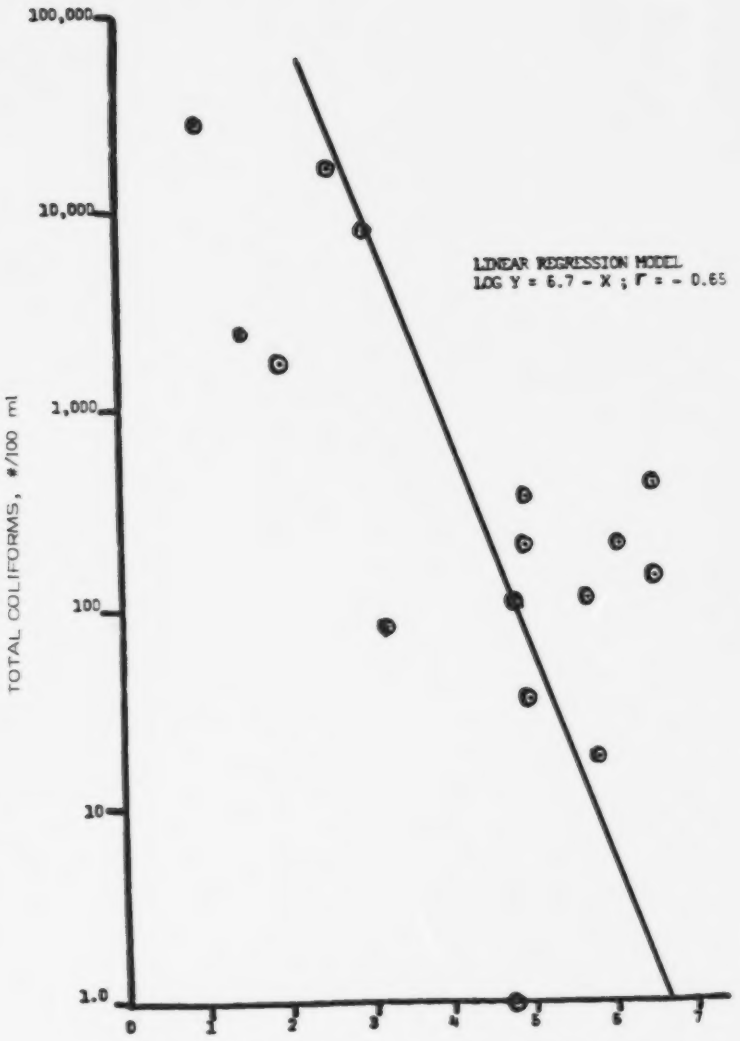

Figure 19. Total coliform number as a function of ozone dosage: UTSD - May 1977.

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In order to achieve planned seed titers (10 pfu/3.8 liters), virus dilutions were performed in the field environment based on an expected original titer value provided by the laboratory supplying the seed stock. Uniformity of original titer is critically important to perform these dilutions correctly; i.e., to avoid too high or too low seed titers. Two examples of low titers occurred, one at the Marlborough Easterly Wastewater Plant, and the second at the Taft Pilot Plant. Both low titer seed samples were diluted to a titer of 70 pfu's in 189 l (50 gal.). The resulting virus titers were too low to provide accurate recovery data for recoveries below 10 percent, as experienced for other plants in this study. Hence, the lack of virus detection at Marlborough and Taft may simply have been due to these low recovery efficiencies and hence large statistical variations.

The medium titer experiments at Marlborough Easterly showed a virus recovery of 34.2% before additional concentration procedures were performed in the laboratory, and 0.7% after these procedures. Polyethylene glycol (PEG) water extraction techniques were used in the latter instance. The poor recovery after PEG extraction is unexplained. PEG extraction has now been discontinued.

The seeded viruses perform as "indicators" of our ability to detect cultured as well as indigenous viruses. However, the recovery percentages refer to the seeded indicator viruses alone. No evidence is presently available to show that the percentage of recovery of seeded viruses is the same for indigenous viruses. Both added virus seed stock and various types of natural viruses from the four treatment plant effluents were recovered in this program. The applicability of these techniques to virus detection has been shown and can be used to study other questions on virus behavior and survival in the environment. Additional work remains to be done in future programs to understand better the wide range of virus recoveries experienced here.

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LEGEND TO APPENDIX A

*pfu	=	Virus isolates measured in plaque-forming units for 50% of the sample on BGM cells and 50% on PMK cells.
P	=	Poliovirus (type 1, 2, or 3).
CA	=	Coxsackievirus A (type 9).
CB	=	Coxsackievirus B (type 1, 2, 3, 4, 5, or 6).
Mixture	=	Greater than one virus identified (not typed by these methods).
Unid.	=	Virus identified (not typed by these methods).
BGM	=	Buffalo Green Monkey Kidney Culture
PMK	=	Africa Green Primary Monkey Kidney Culture

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APPENDIX A

TABLE A-1. UTSD TREATMENT PLANT: COLORADO TERTIARY TREATMENT PLANT SAMPLED PRIOR TO DISINFECTION

Date 1977	Time	Duration (hrs)	Volume (liters)	Viruses (pfu) BGM	Viruses (pfu) PMK	Identification P1	P2	P3	CB1	CB2	CB3	CB4	CB5	Mixture	Unid.	Total virus (pfu/380 l)
4/18	1538	0.5	186	0	0	-	-	-	-	-	-	-	-	-	-	0
4/20	1431	2.0	380	2	1	1	1	1	-	-	-	-	-	-	0	3
4/21	1213	2.5	403	4	0	-	1	1	-	-	1	-	-	-	1	4
4/22	1000	2.0	380	1	0	-	-	-	-	-	-	-	-	-	1	1
4/25	0837	2.1	384	20	3	3	6	1	1	-	-	-	-	-	12	40
4/26	1602	1.8	380	0	0	-	-	-	-	-	-	-	-	-	-	0
4/27	1414	2.1	380	11	4	3	-	-	-	-	-	-	1	-	11	22
4/28	1152	2.5	380	27	0	-	2	-	-	-	-	-	6	-	19	54
4/29	1013	2.7	380	19	0	5	3	2	-	-	-	-	3	-	6	28
5/2	1207	2.0	380	1	1	-	-	-	-	-	-	-	1	-	1	2
5/3	1645	3.9	380	3	0	1	-	-	-	-	-	-	2	-	0	3
5/4	1401	2.3	448	0	0	-	-	-	-	-	-	-	-	-	-	0
5/5	1008	2.5	380	0	1	1	-	-	-	-	-	-	-	-	0	1
5/6	0757	3.0	380	14	2	1	1	-	-	-	8	1	-	-	5	28
5/9	0831	1.8	391	0	0	-	-	-	-	-	-	-	-	-	-	0
5/10	1619	1.9	380	0	0	-	-	-	-	-	-	-	-	-	-	0
			TOTAL	102	12	15	14	5	1	0	9	1	13	0	56	186

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TABLE A-2. UTSD TREATMENT PLANT: COLORADO
TERTIARY TREATMENT PLANT
SAMPLED AFTER DISINFECTION WITH OZONE

Date 1977	Time	Duration (hrs)	Volume (liters)	Viruses (pfu) BGM	Viruses (pfu) PMK	Identification P1	P2	P3	CA9	CB2	CB3	CB4	CB5	Mixture	Unid.	Total Virus (pfu/380 l)
4/19	1159	0.9	190	0	0	-	-	-	-	-	-	-	-	-	-	0
4/20	1427	2.3	380	0	0	-	-	-	-	-	-	-	-	-	-	0
4/21	1231	3.5	380	0	0	-	-	-	-	-	-	-	-	-	-	0
4/22	1023	2.3	380	0	0	-	-	-	-	-	-	-	-	-	-	0
4/25	0843	2.9	380	0	0	-	-	-	-	-	-	-	-	-	7	26
4/26	1620	2.5	380	13	0	3	3	-	-	-	-	-	6	-	1	22
4/27	1404	2.6	380	11	0	2	2	-	-	-	-	-	-	-	30	52
4/28	1204	2.3	380	26	9	3	2	-	-	1	-	-	3	-	4	24
4/29	1003	3.1	380	12	0	1	3	-	-	-	-	-	-	-	-	0
5/2	1209	2.2	380	0	0	-	-	-	-	-	-	-	-	-	-	0
5/3	1641	2.4	380	0	0	-	-	-	-	-	-	-	-	-	2	2
5/4	1408	2.2	350	2	0	-	-	-	1	-	-	-	-	-	0	1
5/5	1000	2.5	380	1	0	-	-	-	-	-	-	-	-	-	1	1
5/6	0821	1.6	380	0	1	-	-	-	-	-	1	-	-	-	0	1
5/9	0827	2.2	380	0	1	-	-	-	-	-	-	-	-	-	-	0
5/10	1617	2.2	380	0	0	-	-	-	-	-	-	-	-	-	-	
			TOTAL	65	11	9	10	0	1	1	1	0	9	0	45	129

1 = Ozone dosage < 3.0 mg/l

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TABLE A-3. MUDDY CREEK WORKS: OHIO
SECONDARY TREATMENT PLANT
SAMPLED PRIOR TO DISINFECTION

Date 1977	Time	Duration (hrs)	Volume (liters)	Viruses (pfu) BGM	Viruses (pfu) PMK	Identification P1	P2	P3	CA9	CB1	CB3	CB4	CB5	Mixture	Unid.	Total Virus (pfu/380 l)
6/27	1518	2.5	380	4	2	3	2	-	-	-	1	-	-	-	-	6
6/28	1235	2.8	243	1	0	-	-	-	-	-	1	-	-	-	-	2
6/29	1034	3.0	315	1	0	-	1	-	-	-	-	-	-	-	-	1
6/30	0848	3.6	361	0	0	-	-	-	-	-	-	-	-	-	-	0
7/1	0600	2.4	380	0	2	1	1	-	-	-	-	-	-	-	-	2
7/5	1627	3.1	270	2	0	-	-	1	-	-	1	-	-	-	-	3
7/6	1827	3.0	323	6	4	1	2	1	-	-	2	1	1	2	-	13
7/7	2000	1.9	380	0	0	-	-	-	-	-	-	-	-	-	-	0
7/8	2203	2.9	220	3	1	-	2	-	-	-	-	-	-	-	2	7
7/18	0017	1.9	380	0	1	-	-	-	-	-	-	-	1	-	-	1
7/19	0218	1.9	380	11	5	-	2	-	-	3	5	2	1	2	1	22
7/20	0402	2.1	380	20	6	2	2	-	1	-	1	2	-	2	16	40
7/21	0634	1.3	296	0	0	-	-	-	-	-	-	-	-	-	-	0
7/22	0806	2.0	380	6	3	1	1	-	-	-	1	2	1	1	2	9
7/23	1000	2.9	384	2	0	-	-	-	-	-	-	1	-	1	-	2
			TOTAL	56	24	8	13	2	1	3	12	8	4	8	21	108

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TABLE A-4. MUDDY CREEK SEWAGE WORKS: OHIO
SECONDARY TREATMENT PLANT
SAMPLED AFTER DISINFECTION WITH CHLORINE

Date 1977	Time	Duration (hrs)	Volume (liters)	Viruses (pfu) BGM	Viruses (pfu) PMK	Identification P1	P2	P3	CB1	CB2	CB3	CB4	CB6	Mixture	Unid.	Total Virus (pfu/380 l)
6/27	1512	2.5	380	3	2	3	2	-	-	-	-	-	-	-	-	5
6/28	1238	2.4	319	0	0	-	-	-	-	-	-	-	-	-	-	0
6/29	1019	3.2	323	0	0	-	-	-	-	-	-	-	-	-	-	0
6/30	0846	2.0	384	0	0	-	-	-	-	-	-	-	-	-	-	0
7/1	0605	2.1	399	0	0	-	-	-	-	-	-	-	-	-	-	0
7/5	1628	2.0	236	1	0	-	-	-	-	-	1	-	-	-	-	2
7/6	1825	3.1	331	4	0	-	-	-	-	-	4	-	-	-	-	5
7/7	2000	3.0	384	0	0	-	-	-	-	-	-	-	-	-	-	0
7/8	2205	3.0	266	0	0	-	-	-	-	-	-	-	-	-	-	0
7/18	0027	1.9	384	2	0	-	1	-	-	-	1	-	-	-	-	2
7/19	0214	3.1	384	0	0	-	-	-	-	-	-	-	-	-	-	0
7/20	0402	1.8	384	0	1	-	-	1	-	-	-	-	-	-	-	1
7/21	0600	1.8	384	0	0	-	-	-	-	-	-	-	-	-	-	0
7/22	0807	2.0	384	1	0	-	-	-	-	-	-	-	1	-	-	1
7/23	0953	1.8	384	0	0	-	-	-	-	-	-	-	-	-	-	0
			TOTAL	11	3	3	3	1	0	0	6	0	1	0	0	16

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TABLE A-5. MUDDY CREEK WORKS AND TAFT PILOT PLANT: OHIO
SECONDARY TREATMENT PLANT
SAMPLED PRIOR TO DISINFECTION

Date 1976	Time	Duration (hrs)	Volume (liters)	Viruses (pfu) BGM	Viruses (pfu) PMK	Identification P1	P2	P3	CB1	CB2	CB3	CB4	CB5	Mixture	Unid.	Total virus (pfu/380 l)
11/22	1445	0.9	190	4	0	-	1	1	-	1	-	1	-	-	-	8
11/29	1200	1.1	380	0	0	-	-	-	-	-	-	-	-	-	-	0
11/30	1000	1.9	380	1	0	-	-	-	-	-	-	1	-	-	-	1
12/1	0800	1.8	380	0	0	-	-	-	-	-	-	-	-	-	-	0
12/2	0600	2.6	380	0	0	-	-	-	-	-	-	-	-	-	-	0
12/3	0400	2.1	319	14	0	-	5	-	-	-	-	3	-	-	6	33
12/6	0200	2.2	334	15	1	1	4	7	-	-	-	4	-	-	-	34
12/6	2400	3.0	380	2	0	-	-	-	-	-	-	2	-	-	-	2
12/7	2200	2.2	380	0	0	-	-	-	-	-	-	-	-	-	-	0
12/8	2000	1.5	380	1	0	-	1	-	-	-	-	-	-	-	-	1
12/9	1745	1.8	380	1	0	-	-	-	-	-	-	1	-	-	-	1
12/10	1600	2.2	380	3	0	1	-	1	-	-	-	1	-	-	-	3
12/13	1400	2.0	380	-	-	-	-	-	-	Concentrate lost			-	-	-	-
12/14	1200	2.8	380	0	0	-	-	-	-	-	-	-	-	-	-	0
12/15	1011	2.6	380	0	0	-	-	-	-	-	-	-	-	-	-	0
			TOTAL	41	1	2	11	9	0	1	0	13	0	0	6	83

TABLE A-6. MUDDY CREEK WORKS AND TAFT PILOT PLANT: OHIO
SECONDARY TREATMENT PLANT
SAMPLED AFTER DISINFECTION WITH OZONE

Date 1976	Time	Duration (hrs)	Volume (liters)	Viruses (pfu) BGM	Viruses (pfu) PMK	Identification P1	P2	P3	CB1	CB2	CB3	CB4	CB5	Mixture	Unid.	Total virus (pfu/380 l)
11/23	1344	0.6	182	0	0	-	-	-	-	-	-	-	-	-	-	0
11/29	1200	1.4	380	-	-	-	-	-	-	Concentrate lost			-	-	-	-
11/30	1000	2.0	380	0	0	-	-	-	-	-	-	-	-	-	-	0
12/1	0800	1.7	380	0	0	-	-	-	-	-	-	-	-	-	-	0
12/2	0600	1.8	380	1	0	-	-	-	-	-	-	1	-	-	-	1
12/3	0400	2.4	380	0	0	-	-	-	-	-	-	-	-	-	-	0
12/6	0233	1.9	380	0	0	-	-	-	-	-	-	-	-	-	-	0
12/6	2400	1.9	380	0	0	-	-	-	-	-	-	-	-	-	-	0
12/7	2200	1.7	380	0	0	-	-	-	-	-	-	-	-	-	-	0
12/8	2012	1.7	380	0	0	-	-	-	-	-	-	-	-	-	-	0
12/9	1800	1.7	380	0	0	-	-	-	-	-	-	-	-	-	-	0
12/10	1600	2.0	384	0	0	-	-	-	-	-	-	-	-	-	-	0
12/13	1423	1.3	380	0	0	-	-	-	-	-	-	-	-	-	-	0
12/14	1215	1.9	384	0	0	-	-	-	-	-	-	-	-	-	-	0
12/15	1015	1.9	414	0	0	-	-	-	-	-	-	-	-	-	-	0
			TOTAL	1	0	0	0	0	0	0	0	1	0	0	0	1

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TABLE A-7. MARLBOROUGH EASTERLY WASTEWATER PLANT: MASSACHUSETTS
SECONDARY TREATMENT PLANT
SAMPLED PRIOR TO DISINFECTION

Date 1977	Time	Duration (hrs)	Volume (liters)	Viruses (pfu) BGM	Viruses (pfu) PHK	Identification P1	P2	P3	CB1	CB2	CB3	CB4	CB5	Mixture	Unid.	Total virus (pfu/380 l)
10/13	1340	0.8	190	0	0	-	-	-	-	-	-	-	-	-	-	0
10/14	1212	1.3	380	1	2	1	1	1	-	-	-	-	-	-	-	3
10/17	1309	1.3	380	1	0	-	-	-	-	-	-	1	-	-	-	1
10/18	1012	1.5	380	0	1	1	-	-	-	-	-	-	-	-	-	1
10/19	0757	1.7	380	0	0	-	-	-	-	-	-	-	-	-	-	0
10/20	0647	1.4	380	1	0	1	-	-	-	-	-	-	-	-	-	1
10/21	0345	1.4	376	0	2	1	-	-	-	1	-	-	-	-	-	2
10/24	1415	1.5	380	0	1	-	1	-	-	-	-	-	-	-	-	1
10/26	1752	1.6	365	0	0	-	-	-	-	-	-	-	-	-	-	0
10/27	1608	1.3	380	0	0	-	-	-	-	-	-	-	-	-	-	0
10/31	1618	1.5	380	0	0	-	-	-	-	-	-	-	-	-	-	0
11/1	1149	1.5	380	0	0	-	-	-	-	-	-	-	-	-	-	0
11/2	1003	1.5	380	0	0	-	-	-	-	-	-	-	-	-	-	0
11/3	0814	1.4	380	0	0	-	-	-	-	-	-	-	-	-	-	0
11/3	1214	1.4	380	0	0	-	-	-	-	-	-	-	-	-	-	0
11/4	0602	1.5	384	0	0	-	-	-	-	-	-	-	-	-	-	0
			TOTAL	3	6	4	2	1	0	1	0	1	0	0	0	9

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TABLE A-8. MARLBOROUGH EASTERLY WASTEWATER PLANT: MASSACHUSETTS
SECONDARY TREATMENT PLANT
SAMPLED AFTER DISINFECTION WITH CHLORINE

Date 1977	Time	Duration (hrs)	Volume (liters)	Viruses (pfu) BGM	Viruses (pfu) PMK	Identification P1	P2	P3	CB1	CB2	CB3	CB4	CB5	Mixture	Unid.	Total virus (pfu/380 l)
10/12	1333	0.6	198	0	0	-	-	-	-	-	-	-	-	-	-	0
10/14	1202	1.9	380	0	0	-	-	-	-	-	-	-	-	-	-	0
10/17	1303	1.6	380	0	0	-	-	-	-	-	-	-	-	-	-	0
10/18	1019	1.4	380	0	0	-	-	-	-	-	-	-	-	-	-	0
10/19	0802	1.3	384	0	0	-	-	-	-	-	-	-	-	-	-	0
10/20	0642	1.2	380	0	0	-	-	-	-	-	-	-	-	-	-	0
10/21	0345	1.2	380	0	0	-	-	-	-	-	-	-	-	-	-	0
10/24	1440	1.2	380	0	0	-	-	-	-	-	-	-	-	-	-	0
10/26	1752	1.3	380	0	0	-	-	-	-	-	-	-	-	-	-	0
10/27	1550	1.2	380	0	0	-	-	-	-	-	-	-	-	-	-	0
10/31	1615	1.3	350	0	0	-	-	-	-	-	-	-	-	-	-	0
11/1	1148	1.4	399	0	0	-	-	-	-	-	-	-	-	-	-	0
11/2	1004	1.2	380	0	0	-	-	-	-	-	-	-	-	-	-	0
11/3	0812	1.3	380	0	0	-	-	-	-	-	-	-	-	-	-	0
11/3	1214	1.3	380	0	0	-	-	-	-	-	-	-	-	-	-	0
11/4	1603	1.2	380	0	0	-	-	-	-	-	-	-	-	-	-	0
			TOTAL	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE A-9. NORTHWEST BERGEN COUNTY WASTEWATER TREATMENT PLANT:
WALDWICK, NEW JERSEY
SECONDARY TREATMENT PLANT
SAMPLED PRIOR TO DISINFECTION

Date 1978	Time	Duration (hrs)	Volume (liters)	Viruses (pfu) BGM	Viruses (pfu) PMK	Identification P1	P2	P3	CB1	CB2	CB3	CB4	CB5	Mixture	Unid.	Total virus (pfu/380 l)
4/13	1255	1.7	399	0	0	-	-	-	-	-	-	-	-	-	-	0
4/14	0916	1.7	399	0	0	-	-	-	-	-	-	-	-	-	-	0
4/19	1509	1.9	399	0	0	-	-	-	-	-	-	-	-	-	-	0
4/21	0924	1.5	399	0	1	-	1	-	-	-	-	-	-	-	-	1
4/24	1405	1.5	399	0	0	-	-	-	-	-	-	-	-	-	-	0
4/25	1225	1.5	399	1	0	1	-	-	-	-	-	-	-	-	-	1
4/26	1030	1.5	399	0	0	-	-	-	-	-	-	-	-	-	-	0
4/27	0848	1.6	399	2	0	-	2	-	-	-	-	-	-	-	-	2
4/27	1232	1.6	399	1	0	1	-	-	-	-	-	-	-	-	-	1
4/28	0907	1.7	399	2	0	-	1	1	-	-	-	-	-	-	-	2
5/3	1555	1.6	399	0	0	-	-	-	-	-	-	-	-	-	-	0
5/4	1100	1.6	399	0	0	-	-	-	-	-	-	-	-	-	-	0
5/5	0852	1.6	399	1	0	1	-	-	-	-	-	-	-	-	-	2
5/8	1220	1.6	399	1	1	1	-	-	-	-	-	-	-	-	1	1
5/9	1241	1.6	399	4	1	-	2	3	-	-	-	-	-	-	-	5
5/11	1246	0.8	190	10	2	-	3	1	-	-	-	-	5	2	1	40
			TOTAL	22	5	4	9	5	0	0	0	0	5	2	2	55

TABLE A-10. NORTHWEST BERGEN COUNTY SEWAGE TREATMENT PLANT
SECONDARY TREATMENT PLANT
SAMPLED AFTER DISINFECTION WITH UV LIGHT

Date 1978	Time	Dur-tion (hrs)	Volume (liters)	Viruses (pfu) BGM	Viruses (pfu) PMK	Identification P1	P2	P3	CB1	CB2	CB3	CB4	CB5	Mix-ture	Unid.	Total virus (pfu/380 l)
4/13	1256	2.1	399	1	0	1	-	-	-	-	-	-	-	-	-	1
4/14	0910	1.6	399	0	0	-	-	-	-	-	-	-	-	-	-	0
4/19	1503	2.0	372	1	0	-	-	-	-	-	-	-	-	-	1	1
4/21	0924	1.5	399	0	0	-	-	-	-	-	-	-	-	-	-	0
4/24	1405	1.5	399	0	0	-	-	-	-	-	-	-	-	-	-	0
4/25	1221	1.6	399	0	0	-	-	-	-	-	-	-	-	-	-	0
4/26	1030	1.5	399	0	0	-	-	-	-	-	-	-	-	-	-	0
4/27	0848	1.5	399	0	0	-	-	-	-	-	-	-	-	-	-	0
4/27	1232	1.6	399	0	0	-	-	-	-	-	-	-	-	-	-	0
4/28	0907	1.7	399	0	0	-	-	-	-	-	-	-	-	-	-	0
5/3	1545	2.3	334	0	0	-	-	-	-	-	-	-	-	-	-	0
5/4	1100	1.6	399	0	0	-	-	-	-	-	-	-	-	-	-	0
5/5	0852	1.6	399	0	0	-	-	-	-	-	-	-	-	-	-	0
5/8	1220	1.7	399	0	0	-	-	-	-	-	-	-	-	-	-	0
5/9	1241	1.5	399	0	0	-	-	-	-	-	-	-	-	-	-	0
5/10	1311	0.9	190	0	0	-	-	-	-	-	-	-	-	-	-	0
			TOTAL	2	0	1	0	0	0	0	0	0	0	0	1	2

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APPENDIX B

VIRUS RECOVERIES FROM SEEDED POLIOVIRUS RUNS

TABLE B-1. MUDDY CREEK WORKS AND TAFT PILOT PLANT: OHIO
SECONDARY TREATMENT PLANT
SUMMARY OF DATA COLLECTED/ANALYZED FROM SEEDED EFFLUENT SAMPLES

Date	Sample point	Sample type	Time	Duration (hrs)	Volume Sampled (liters)	Measured input seed, pfu*	Viruses recovered pfu*	Background virus pfu*	Percent Recovery	Remarks
11/22	A	Medium titer	1647	N.A.	190	2800 (15/1)				Seed input to secondary effluent
	A	Medium titer	1647	0.9	190		69 (.36/1)		2.3	Secondary effluent
	A	Medium titer	1445	0.9	190			4 (0.02/1)		Background
11/23	B	Low titer	1351	N.A.	194	68 (0.35/1)				Seed input to disinfected secondary effluent
	B	Low titer	1351	1.0	194		0		0	Disinfected secondary effluent (ozone)
	B	Low titer	1344	0.6	182			0		Background

*Plaque-forming units per sample
**Corrected for background

TABLE B-2. MUDDY CREEK WORKS: OHIO
SECONDARY TREATMENT PLANT
SUMMARY OF DATA COLLECTED/ANALYZED FROM SEEDED EFFLUENT SAMPLES

Date 1977	Sample point	Sample type	Time	Duration (hrs)	Volume Sampled (liters)	Measured input seed, pfu*	Viruses recovered pfu*	Background virus pfu*	Percent recovery**	Remarks
6/23	A	Medium titer	1335	N.A.	190	28,000 (147/1)				Seed input to secondary effluent
	A	Medium titer	1335	1.3	190		940 (5/1)		1.7	Secondary effluent
	A	Medium titer	1355	1.3	190			470 (3/1)		Background
6/22	B	Low titer	1401	N.A.	190	5,200 (27/1)				Seed input to disinfected secondary effluent
	B	Low titer	1401	1.3	190		4,700 (25/1)		46.2	Disinfected secandary effluent (chlorine)
	B	Low titer	1401	1.3	190			2,300 (12/1)		Background

*Plaque-forming units per sample
**Corrected for background

TABLE B-3. UTSD TREATMENT PLANT: COLORADO
TERTIARY TREATMENT PLANT
SUMMARY OF DATA COLLECTED/ANALYZED FROM SEEDED EFFLUENT SAMPLES

Date 1977	Sample point	Sample type	Time	Duration (hrs)	Volume Sampled (liters)	Measured input seed, pfu*	Viruses recovered pfu*	Background Virus pfu*	Percent recovery**	Remarks
4/18	A	Medium titer	1541	N.A.	179	11,000 (6/1)				Seed input to tertiary effluent
	A	Medium titer	1541	0.5	179		1,100 (6/1)		9.9	Tertiary effluent
	A	Medium titer	1538	0.5	180			0		Background
4/19	B	Low titer	1556	N.A.	198	1,400 (7/1)				Seed input to disinfected tertiary effluent
	B	Low titer	1556	1.0	198		110 (0.6/1)		7.7	Disinfected tertiary effluent
	B	Low titer	1159	0.9	190			0		Background

*Plaque-forming units per sample
**Corrected for background

TABLE B-4. MARLBOROUGH EASTERLY WASTEWATER PLANT: MASSACHUSETTS
SECONDARY TREATMENT PLANT
SUMMARY OF DATA COLLECTED/ANALYZED FROM SEEDED EFFLUENT SAMPLES

Date 1977	Sample point	Sample type	Time	Duration (hrs)	Volume Sampled (liters)	Measured input seed, pfu*	Viruses recovered pfu*	Background Virus pfu*	Percent recovery**	Remarks
10/13	A	Medium titer	1352	N.A.	163	2,000 (12/1)				Seed input to secondary effluent
	A	Medium titer	1352	1.1	163		13 (0.08/1)		0.7	Secondary effluent
	A	Medium titer	1340	0.8	190			0		Background
10/12	B	Low titer	1333	N.A.	175	71 (0.4/1)				Seed input to disinfected secondary effluent
	B	Low titer	1333	0.8	175		0		0	Disinfected secondary effluent (chlorine)
	B	Low titer	1333	0.6	198			0		Background

*Plaque-forming units per sample
**Corrected for background

TABLE B-5. NORTHWEST BERGEN COUNTY
SECONDARY WASTEWATER TREATMENT PLANT: NEW JERSEY
SUMMARY OF DATA COLLECTED/ANALYZED FROM SEEDED EFFLUENT SAMPLES

Date 1978	Sample point	Sample type	Time	Duration (hrs)	Volume Sampled (liters)	Measured input seed, pfu*	Viruses recovered pfu*	Background Virus pfu*	Percent recovery**	Remarks
5/11	A	Medium titer	1246	N.A.	190	40,000 (211/l)				Seed input to secondary effluent
	A	Medium titer	1246	0.8	190		5,800 (31/l)		14.5	Secondary effluent
	A	Medium titer	1246	0.8	190			12 (0.06/l)		Background
5.10	B	Low titer	1311	N.A.	190	6,200 (33/l)				Seed input to disinfected secondary effluent
	B	Low titer	1311	0.8	190		4,209 (0.44/l)		68	Disinfected secondary effluent (ultraviolate)
	B	Low titer	1311	0.9	190			0		Background

*Plaque-forming units per sample
**Corrected for background

TABLE C-1. TREATMENT PLANT EFFLUENT CHARACTERISTICS

		Pre total coliform (Number/ 100 mg)	Post total coliform (Number/ 100 mg)	Pre fecal coliform (Number/ 100 mg)	Post fecal coliform (Number/ 100 mg)	Pre TSS (mg/l)	Post TSS (mg/l)	Pre COD (mg/l)	Post COD (mg/l)
NW Bergen	$\bar{x}$	1.8×10^5	37.2	4.1×10^4	23.6	5.3	5.9	30.2	26.7
	Sn	1.4×10^5	21.0	4.5×10^4	12.9	2.7	2.0	3.9	4.4
UTSD	$\bar{x}$	8.4×10^5	3.8×10^3	6.9×10^5	3.3×10^3	8.5	4.8	36.1	35.3
	Sn	1.1×10^6	7.7×10^3	1.1×10^6	6.55×10^3	5.5	5.8	8.6	18.6
	Sn-1	1.2×10^6	8.0×10^3	1.1×10^6	6.8×10^3	5.7	6.0	8.9	19.3
Muddy Creek	$\bar{x}$	1.65×10^5	6.5×10^3	7.8×10^4	1.0×10^3	2.7	2.0	27.9	25.0
	Sn	1.5×10^5	1.0×10^4	1.1×10^5	2.7×10^3	1.0	1.1	5.4	5.3
Marlborough	$\bar{x}$	1.8×10^4	2.3×10^2	1.8×10^4	8.4	12.6	10	24.4	-
	Sn	1.9×10^4	3.1×10^2	1.9×10^4	7.4	3.7	-	6.8	-
Muddy Creek/Taft	$\bar{x}$	7.8×10^5	3.1×10^3	1.9×10^5	1.2×10^3	7.4	5.9	39.2	33.4
	Sn	5.1×10^5	5.9×10^3	1.2×10^5	6.1×10^3	7.0	4.1	14.1	9.1

$\bar{x}$ = Mean
Sn = Standard deviation
Pre = before disinfection
Post= after disinfection

TABLE C-2. WASTEWATER TREATMENT PLANT EFFLUENT CHARACTERISTICS

		Pre TOC (mg/l)	Post TOC (mg/l)	Pre turbidity (NTU)	Post turbidity (NTU)	Disinfectant Dosage (mg/l)	Disinfectant Residual (mg/l)	Contact time (min)
NW Bergen	$\bar{x}$	13.0	13.1	2.1	2.2	131,000 ($UW\text{-}sec/cm^2$)		.06
	Sn	1.4	0.8	0.7	0.7	-	-	-
UTSD	$\bar{x}$	-	-	5.0	2.2	4.2	-	37
	Sn	-	-	1.3	1.0	2.8	-	-
Muddy Creek	$\bar{x}$	11.6	10.9	1.8	2.0	-	1.2	51
	Sn	4.3	4.1	0.5	0.4	-	0.4	-
Marlborough	$\bar{x}$	6.5	-	5.0	6.45	-	1.3	47
	Sn	2.9	-	1.1	2.4	-	0.4	-
Muddy Creek/Taft	$\bar{x}$	11.2	10.3	4.0	2.4	7.9	0.13	.5
	Sn	6.0	4.7	3.9	1.3	3.2	0.007	-

$\bar{x}$ = Mean
Sn = Standard deviation
Pre = before disinfection
Post = after disinfection